Advances in HD
Bio-Artificial Kidney (BAK)

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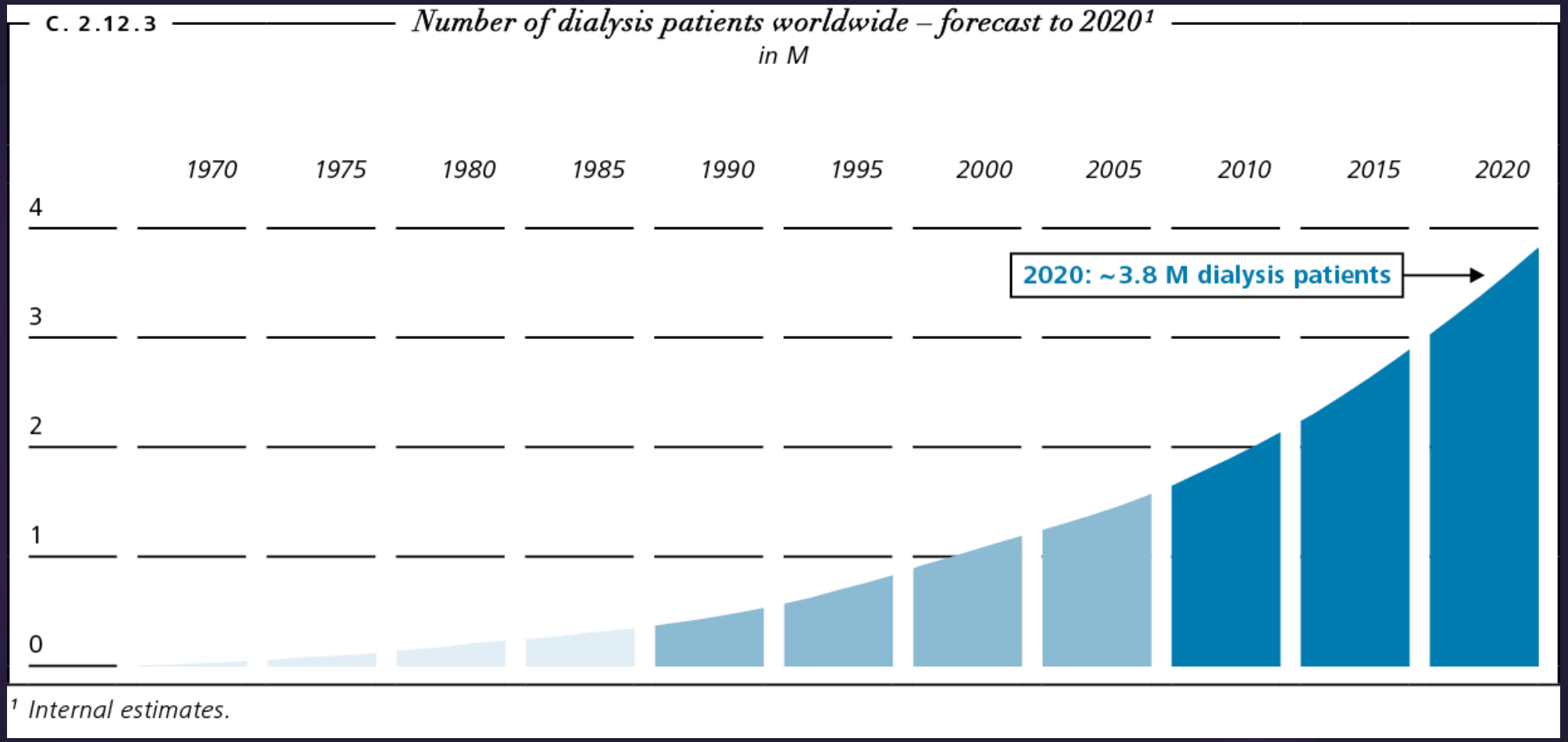
AIN SHAMS UNIVERSITY

Do we Need Innovations in HD ?

- 2.5 millions on HD
- 350 million HD sessions / year



Questions to be Addressed

Do we Need Innovations in HD Therapy ?

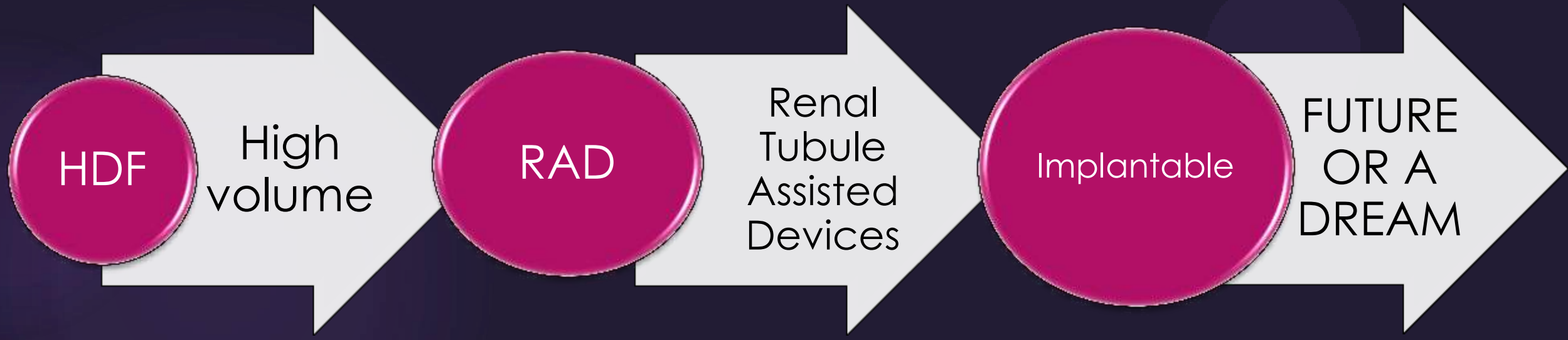
Are patients satisfied by HD ?

What is the Best marker of HD adequacy for the Nephrologist ?

Current HD Therapy limitations

- Therapy only depends on Filtration
- Completely retention of PB uremic toxins
- No single or multiple Biomarkers are sufficient .
- Problems in Inflammation and Malnutrition.
- Non Physiological HD frequency and duration.

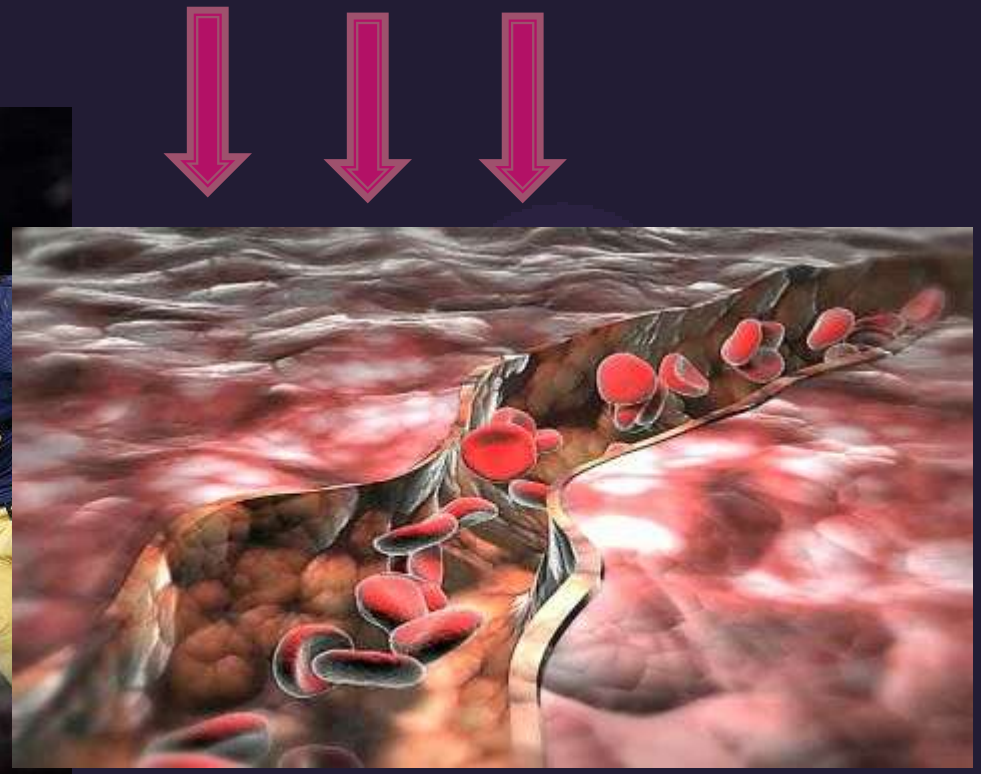
Advances in HD therapy



The uremic syndrome (the lancet Mar 2, 2016)

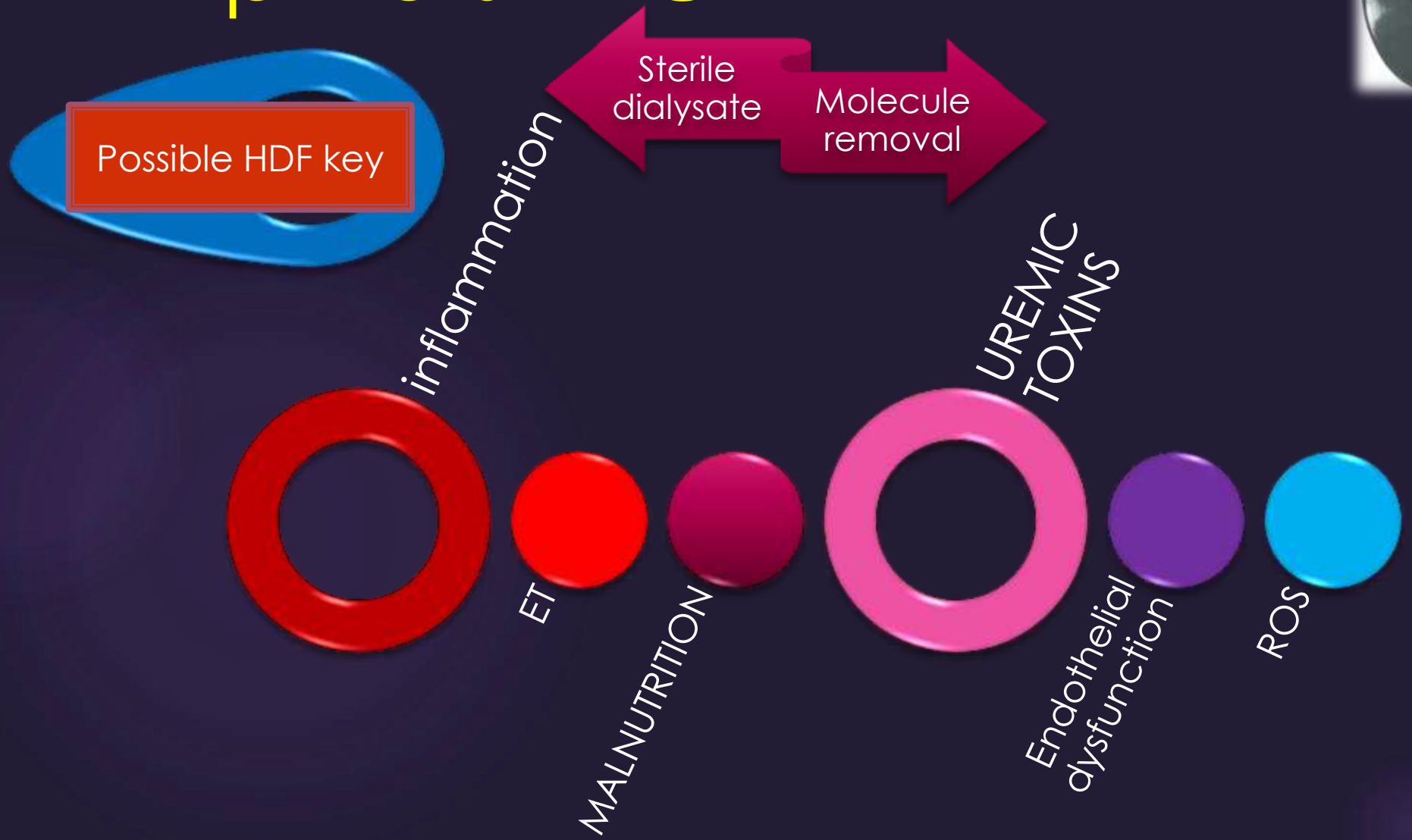
CKD MANY PLAYERS WITH THE SAME TARGET

Targeting

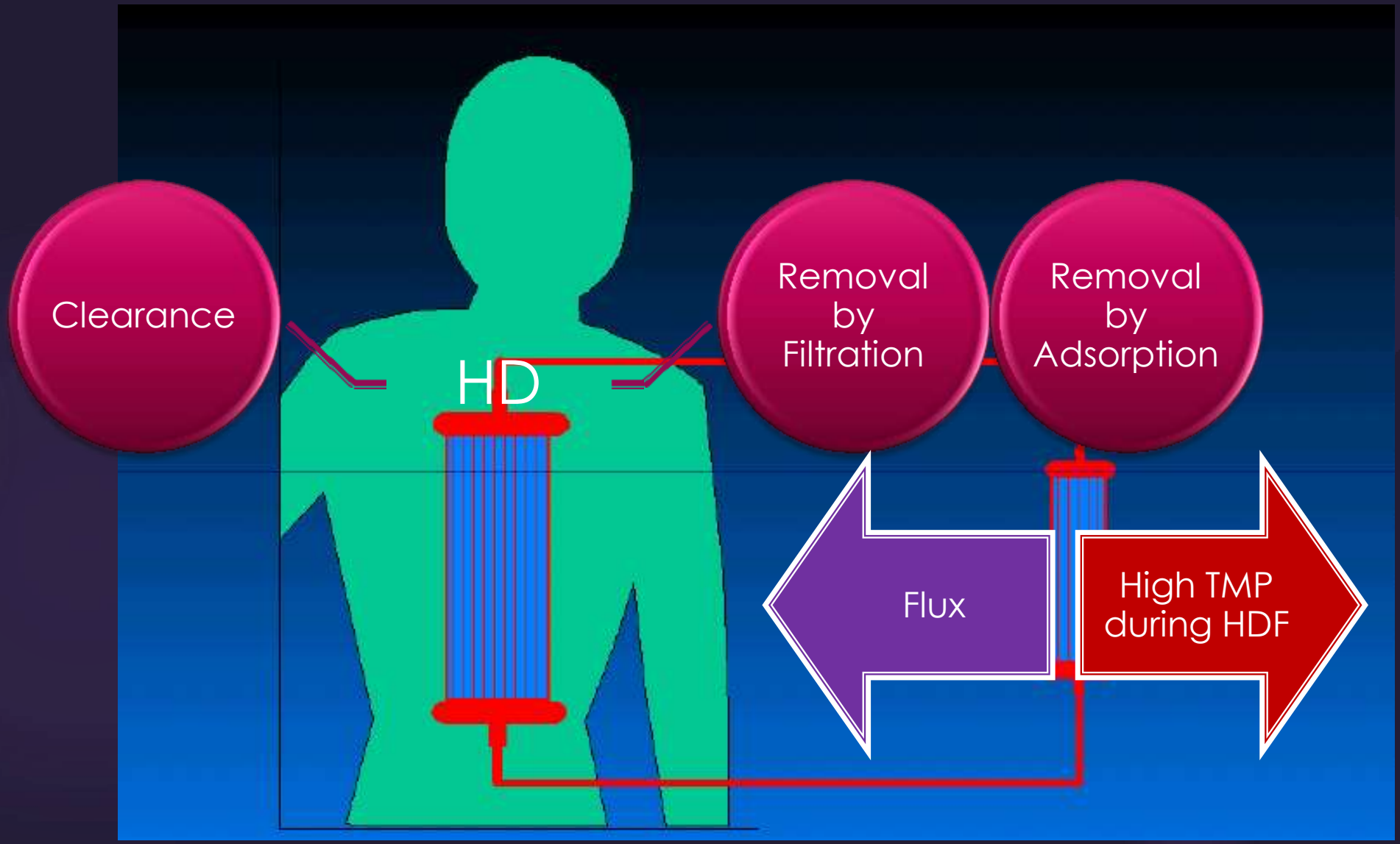


Inflamed ET

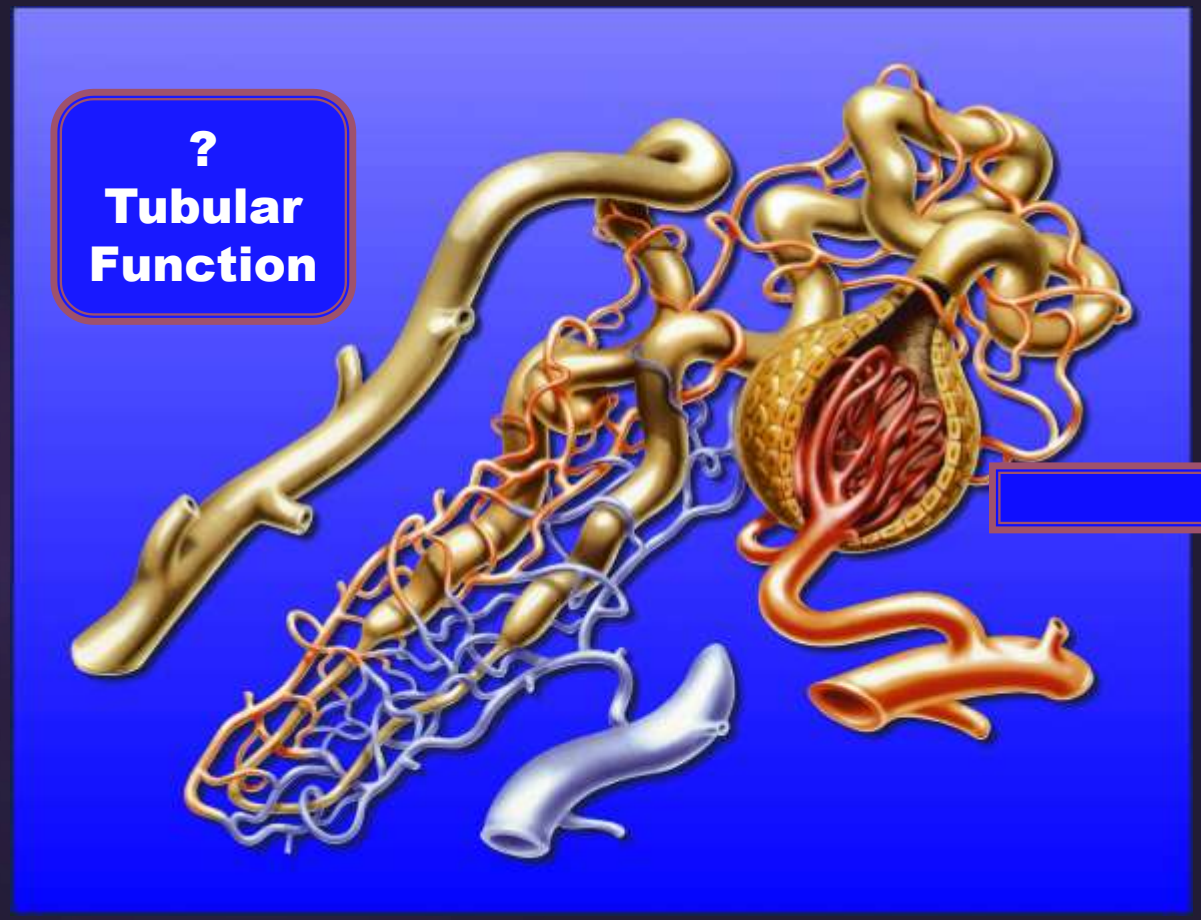
Amplifiers in CKD



HD : a Filtration Therapy

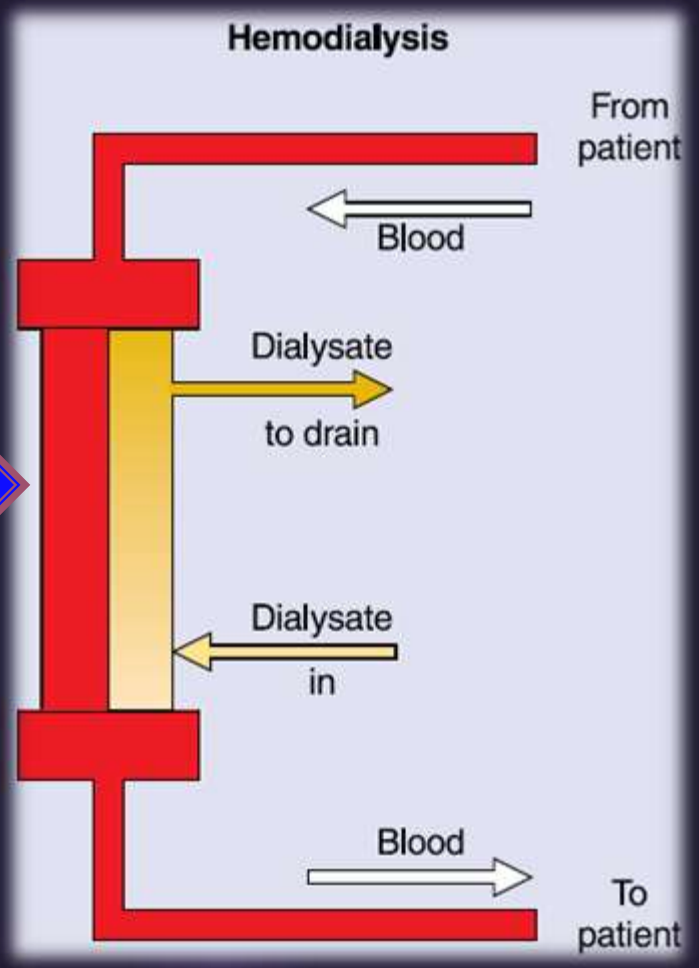


Filtration Versus Metabolism



Current HD

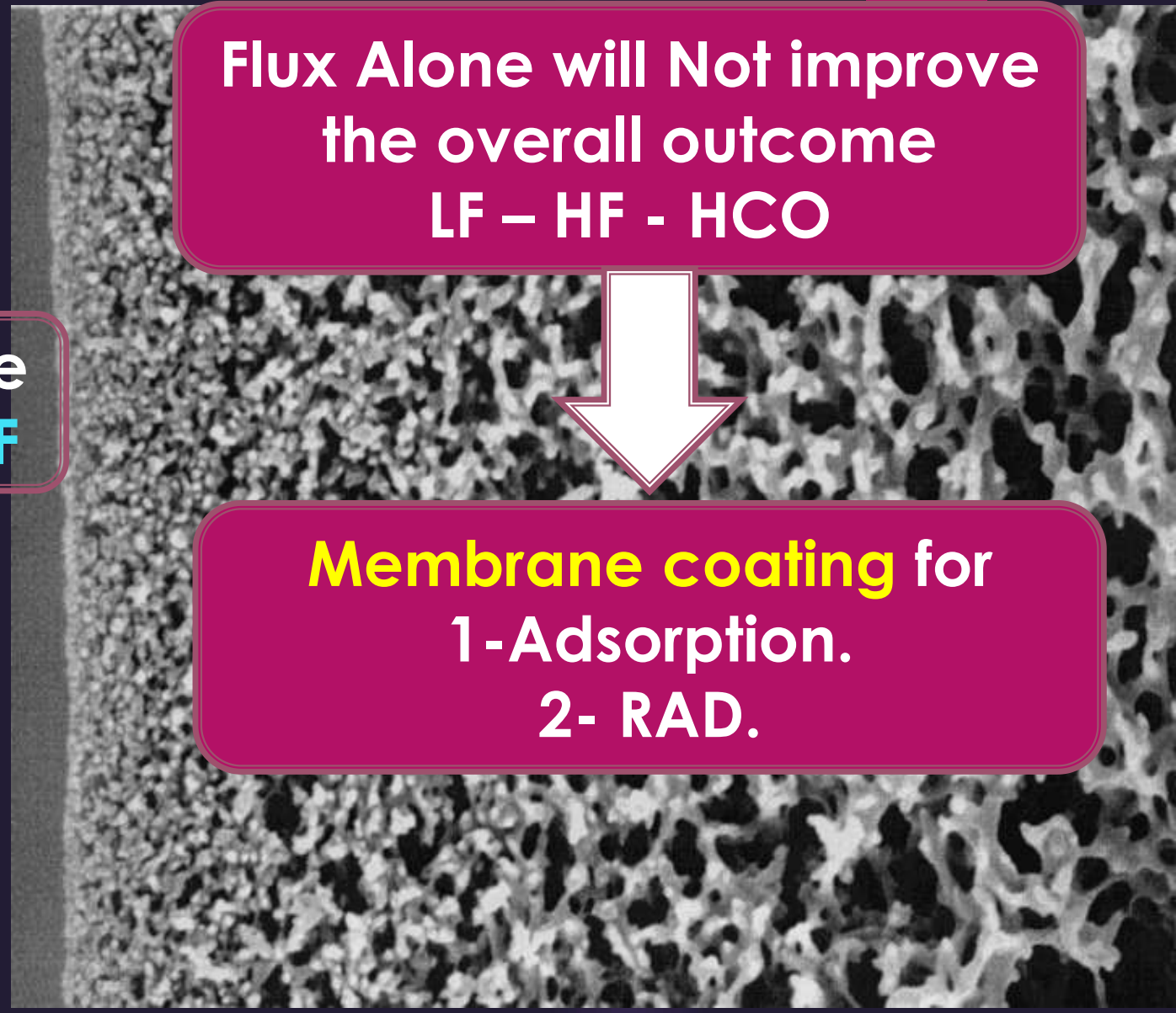
Filtration Function



Researches in HD membranes Towards better sieving

- SC
- Antifouling
- Larger Pores
- Accepting 3 gm Albumin loss / session

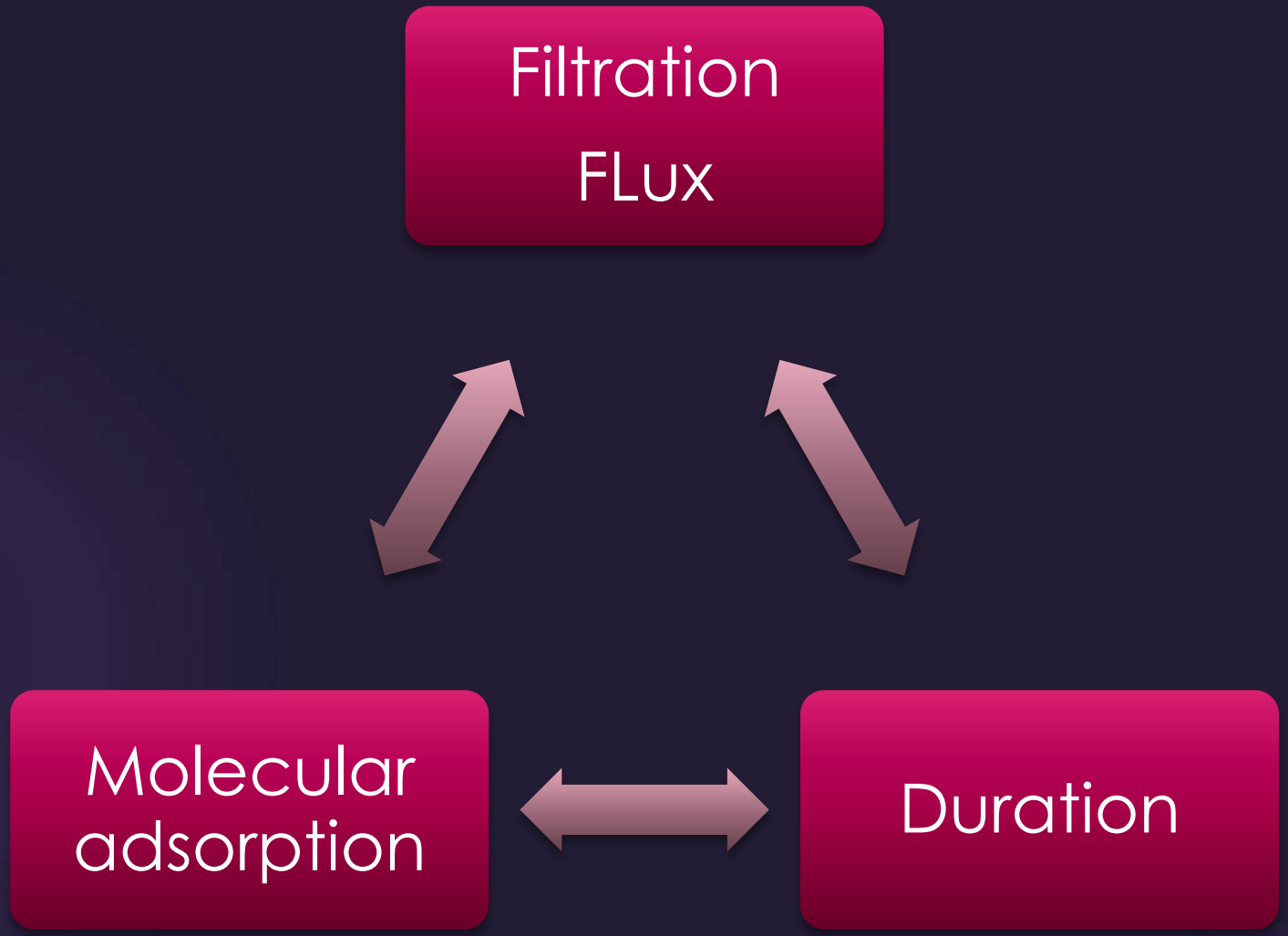
Uremic toxins and Pore diameter Is Not a Simple Button and a Hole



Flux Alone will Not improve the overall outcome
LF – HF - HCO

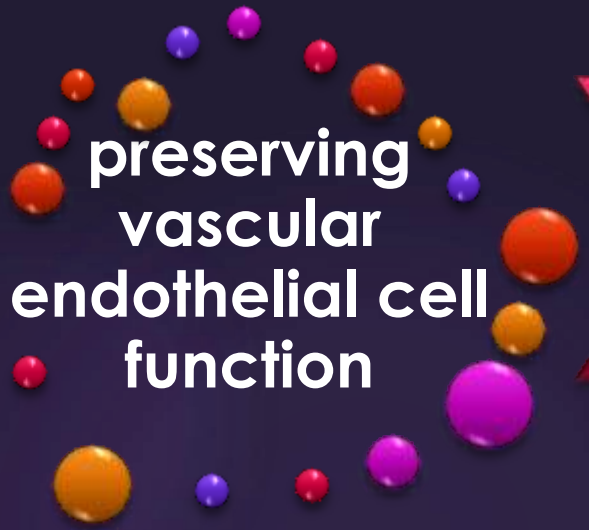
Membrane coating for
1-Adsorption.
2- RAD.

Removal By filtration



Therapeutic challenges in Reversing the Attack of the Endothelial cells

HDF Japanese Perspective Blood Purif 2015;40



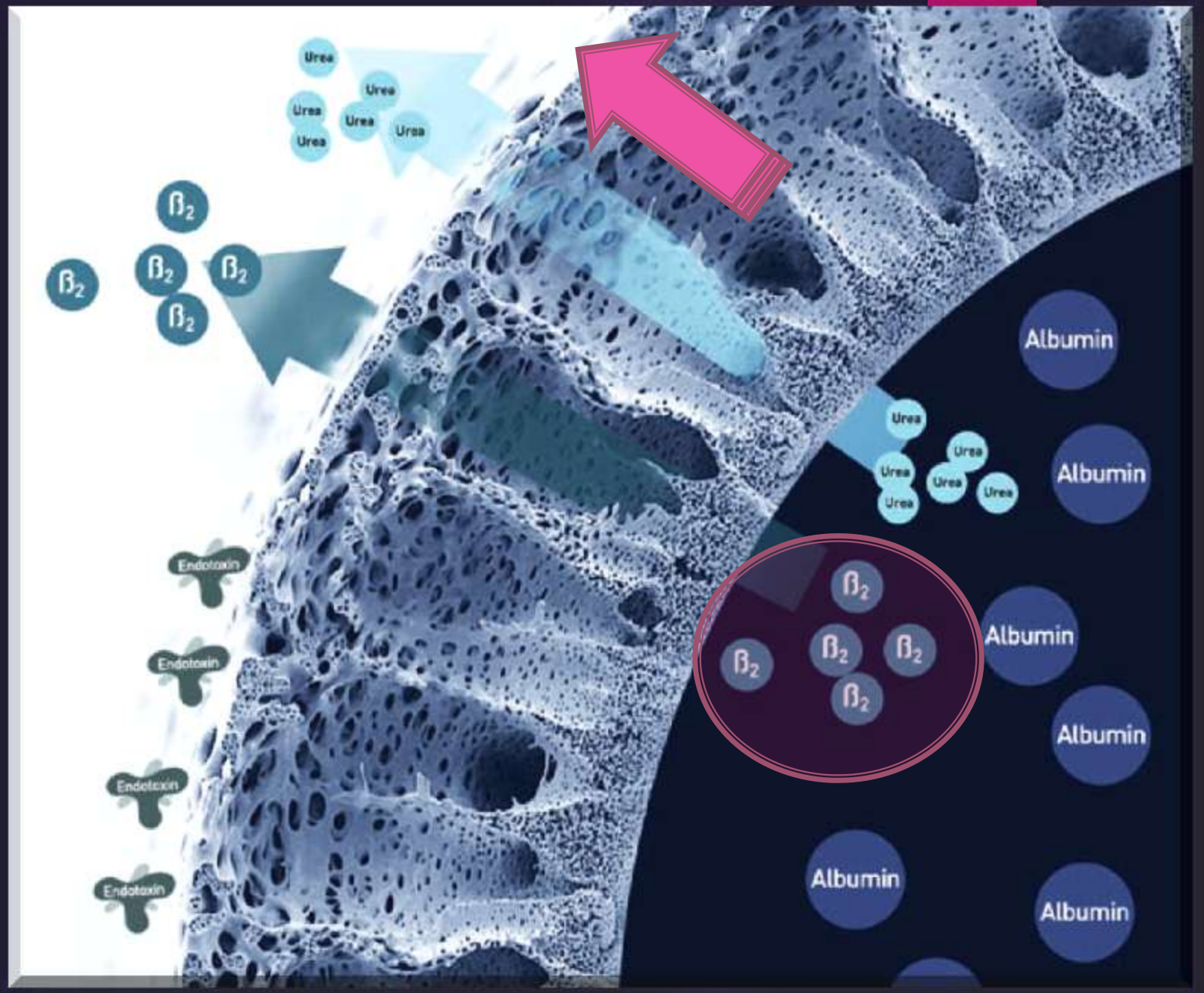
Nephrol Dial Transplant 2014; 29:

reduction in fibroblast growthfactor-23

improvement of the nutritional status by reducing inflammatory stress

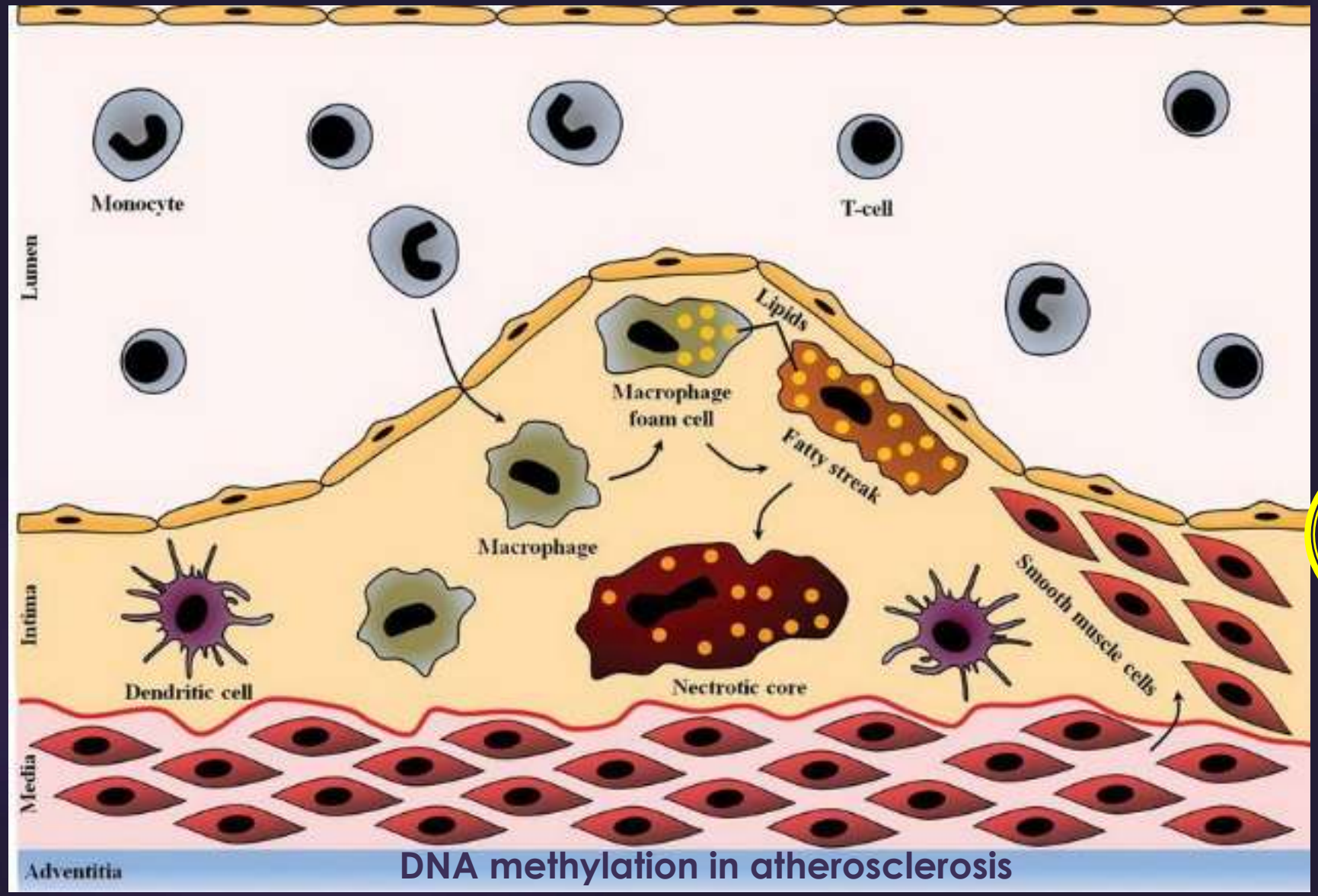
HD and HDF is considered to be Glomerular Replacement therapy GRR

Focusing in Middle Molecules



HDF and Endothelia Functions versus HF dialysis

- OLHDF improved endothelial dysfunction
- decrease in the number of endothelial microparticles (EMP)
- increase in the percentage of endothelial progenitor cells (EPC)



DNA methylation in atherosclerosis

monocytes differentiate into macrophages

gene-specific DNA methylation suggests alterations and advanced atherosclerotic lesions

toxic uraemic milieu may exert a crucial impact on epigenetic gene regulation and CKD-associated accelerated arteriosclerosis

Adam M. Zawada et al. Nephrol. Dial. Transplant. 2013

WHICH MEMBRANE FOR HDF

Wider inner diameter ≥
200 um

Higher UF ≥ 50

Higher Blood Flow ≥ 400 ml/min

Substitution volume

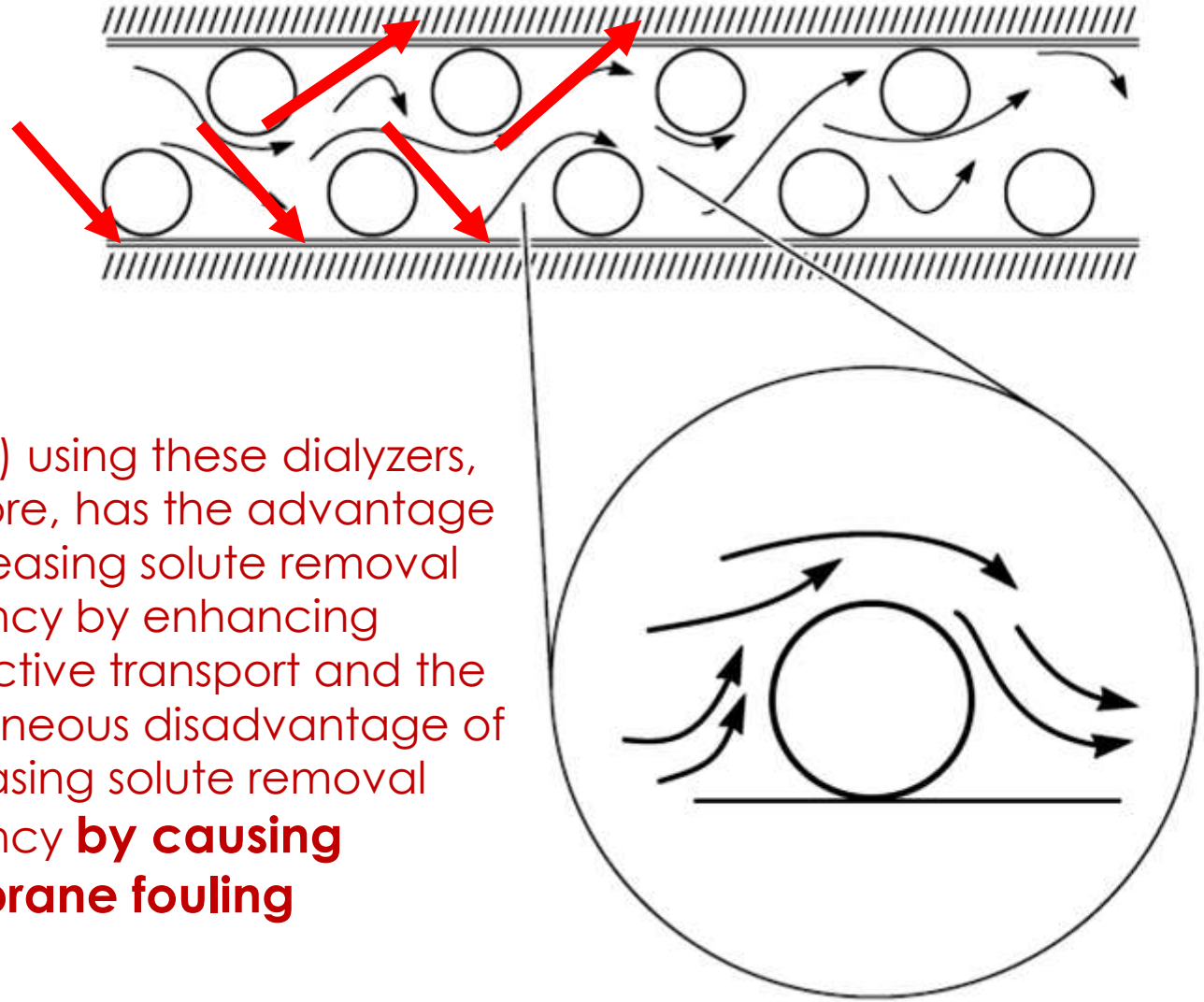
25 litre

SC of B2m

≥ 0.8

Inner diameter Fiber ≤ 200 um

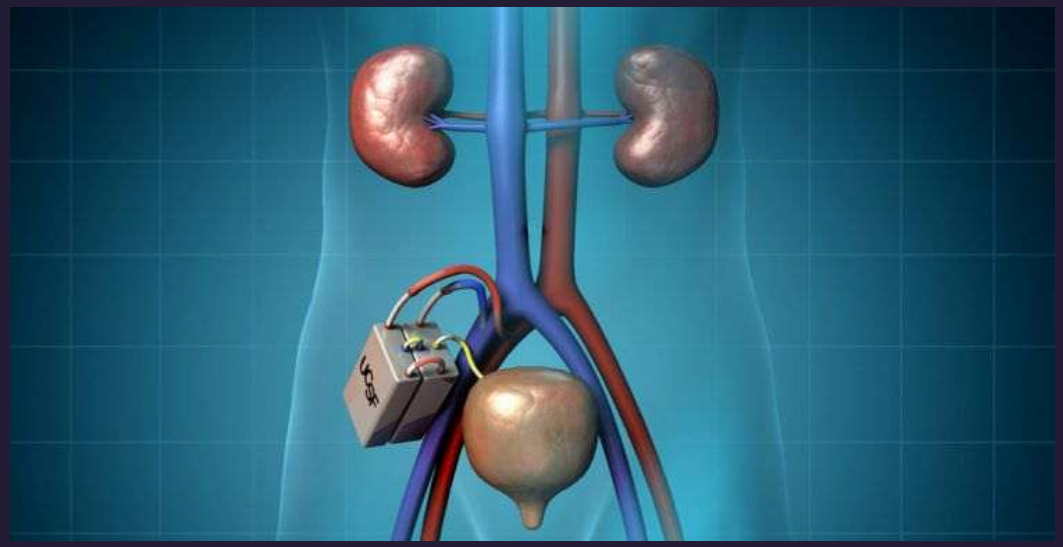
Contrib Nephrol. 2011;173



(IFEHD) using these dialyzers, therefore, has the advantage of increasing solute removal efficiency by enhancing convective transport and the simultaneous disadvantage of decreasing solute removal efficiency **by causing membrane fouling**

TURBELANT FLOW HIGH SHEAR STRESS INCREASE FOILUNG INSIDE THE FIBER LUMEN

If we believe in History : we should Realize The Future !



BioArtificial Kidney
Implantable IBAK

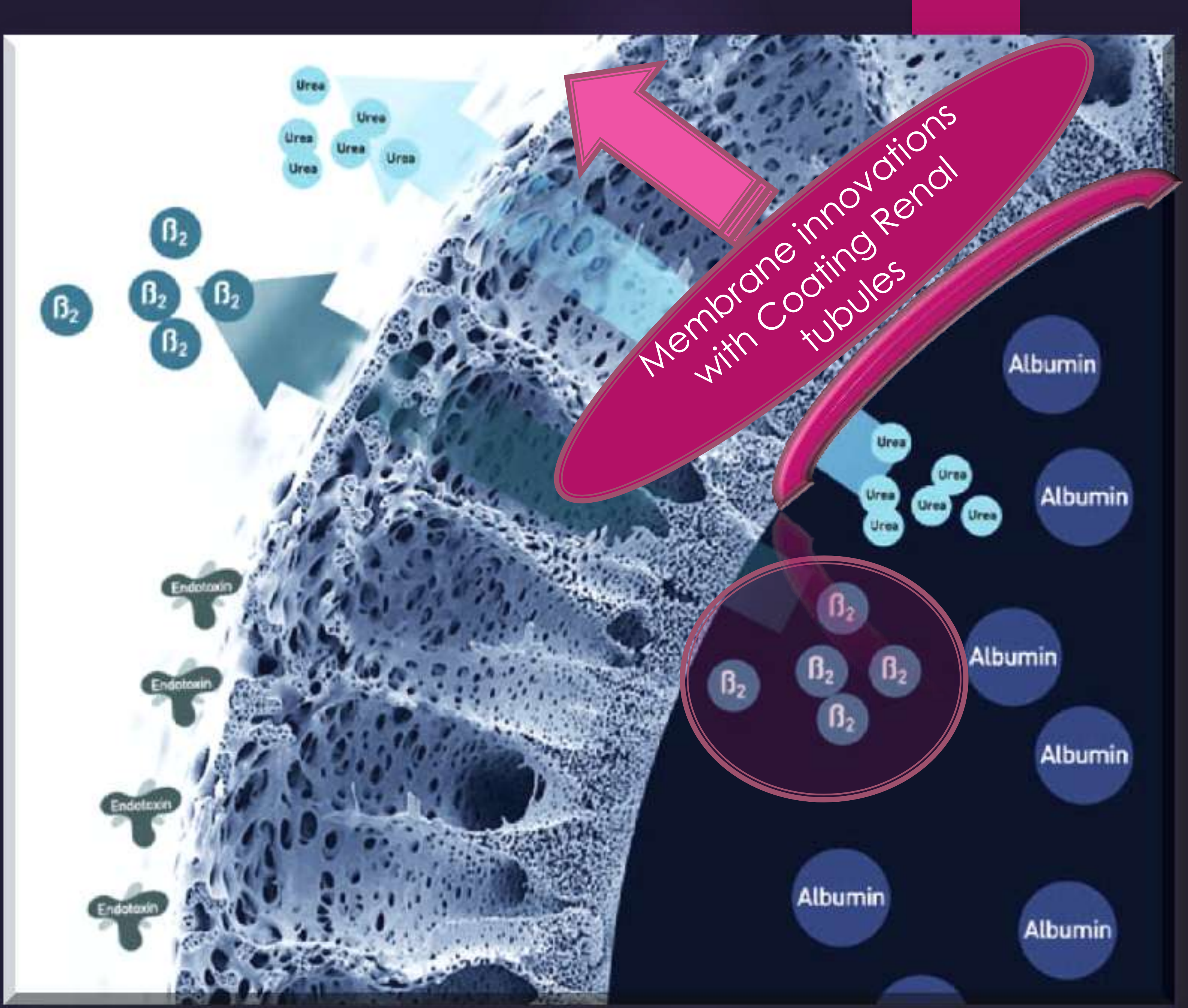
Renal Tubule Assisted Devices

The Philosophy Deserve to innovate

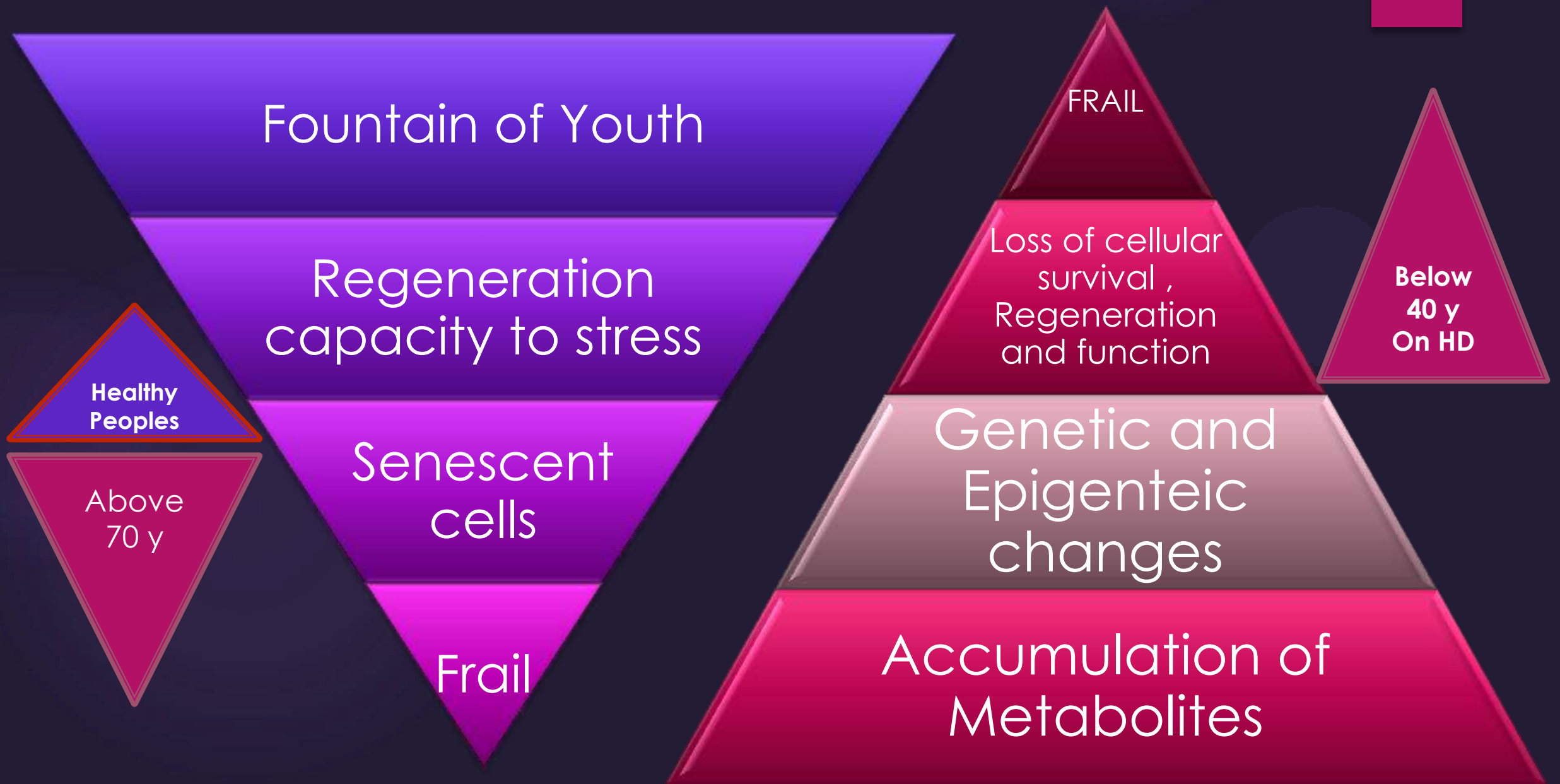
Why we are in need of RAD ?

HD is considered to be Glomerular Replacement therapy GRR

Focusing in Middle Molecules

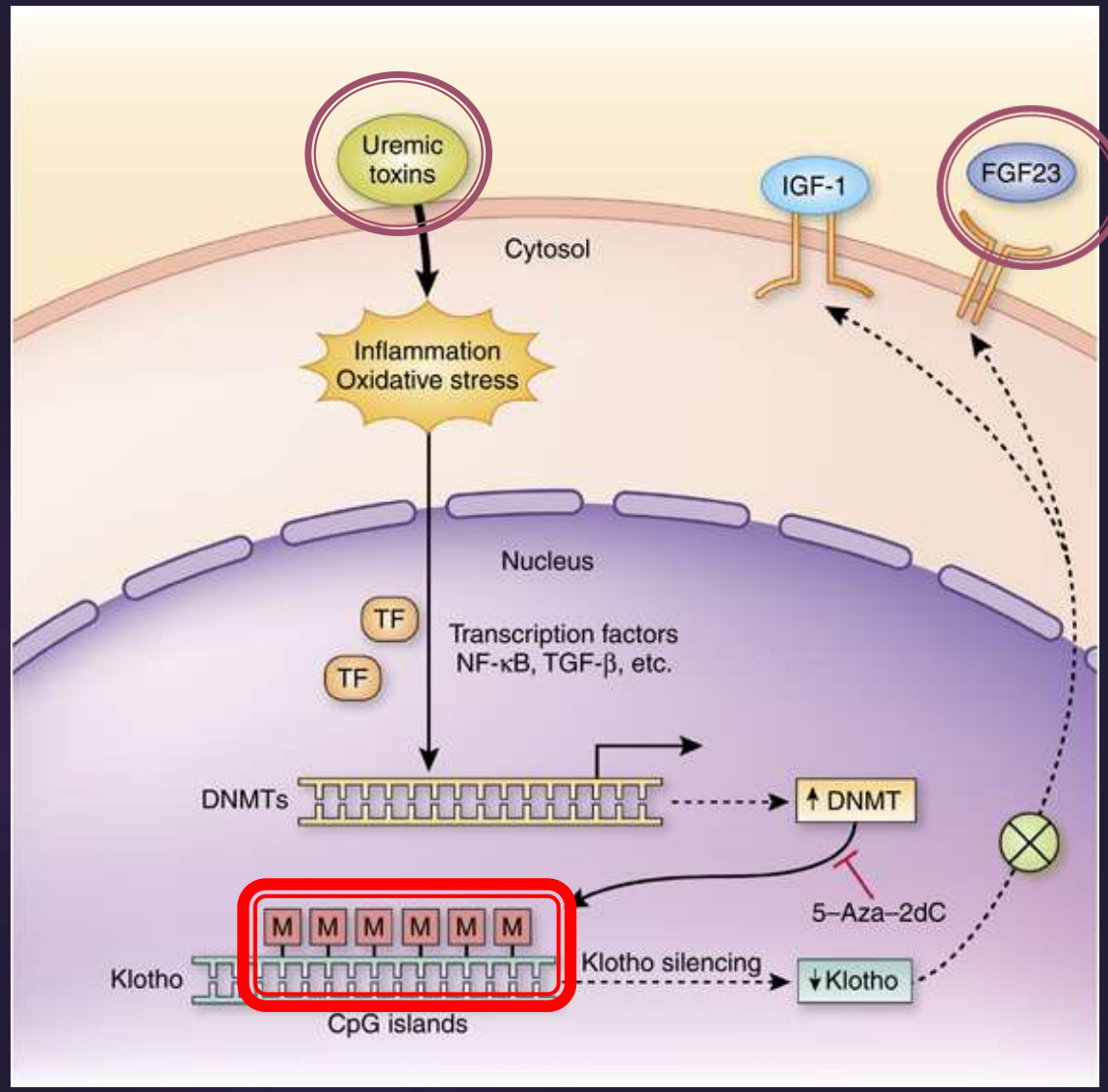


What Beyond Filtration is Missing in HD



ROLE OF UREMIC TOXINS : Effect on KLOTHO Gene

JASN December 4, 2014



coreceptor

Uremic Toxins

induce DNA methyltransferase (DNMT) protein expression,

Hypermethylated status of *KLOTHO*

Nephrol Dial Transplant (2013) 28: 48–54
doi: 10.1093/ndt/gfs451
Advance Access publication 8 November 2012

Out of control: accelerated aging in uremia

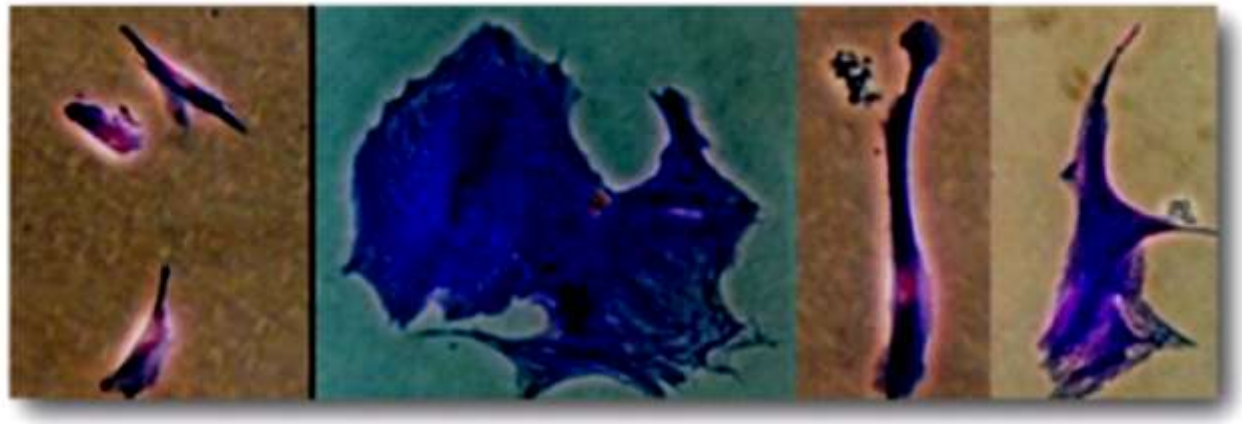
Jeroen P. Kooman[1], Natascha J.H. Broers[1], Len Usvyat[2], Stephan Thijssen[2], Frank M. van der Sande[1], Tom Cornelis[1], Nathan W. Levin[2], Karel M.L. Leunissen[1] and Peter Kotanko[2]

[1]Department of Internal Medicine, Division of Nephrology, University Hospital Maastricht, Maastricht, the Netherlands and [2]Renal Research Institute, New York, USA

reduced physical capacity and the loss of muscle mass are associated with a high prevalence of frailty. Phenotypically, frailty is a clinical syndrome characterized by multiple pathologies, such as weight loss, fatigue, weakness, low activity, slow motor performance and balance and gait abnormalities

44% of dialysis patients below 40 years of age were classified as frail

Advanced CKD characterized by many features of Aging:

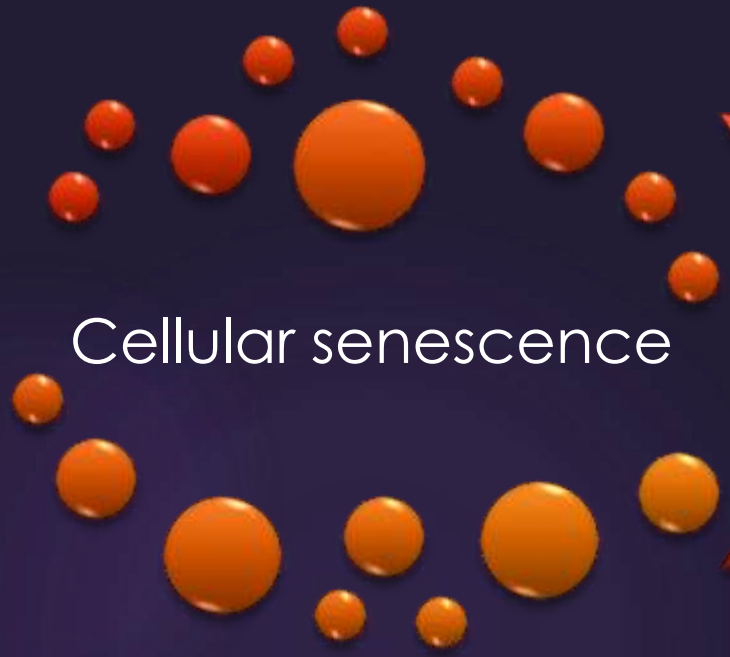


Senescent cells change their morphological characteristics and these changes are accompanied by alterations in nuclear structure, gene expression, protein processing and metabolism.

Senescent cells

Stem cells : Senescent

Cellular senescence is a state of permanent and irreversible cell cycle arrest American Journal of Phys 15 July 2012



Cellular senescence

reduced capability to respond to stress

irreversible growth arrest,

express a different set of genes as P53

P16 – P21

insufficient regenerative capacity of organs

Main activator of Senescence

inhibit the cell cycle progression,

National Kidney Foundation®
Journal of Renal Nutrition

Indoxyl Sulfate Induces Endothelial Cell Senescence by Increasing Reactive Oxygen Species Production

January 2012Volume 22, Issue 1

Protein-Bound Uremic Toxins: New Culprits of Cardiovascular Events in Chronic Kidney Disease Patients

toxins

Toxins **2014**, *6*, 665-678

Cardiorenal Syndrome

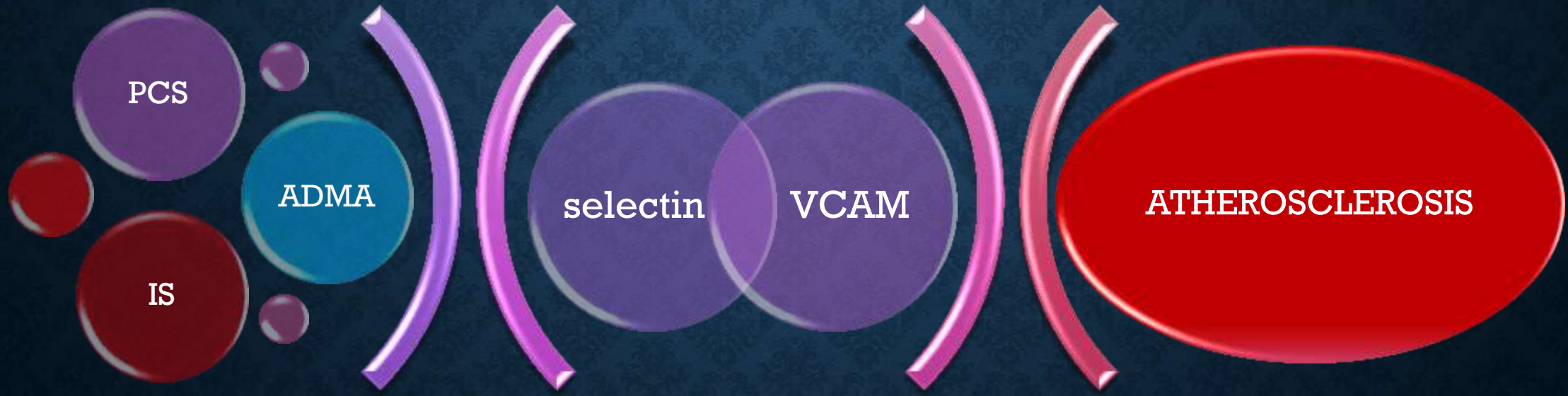
The Emerging Role of Protein-Bound Uremic Toxins

American Heart Association

Circulation Research November 9, 2012

Protein-Bound Uremic Toxins Stimulate Crosstalk between Leukocytes and Vessel Wall

JASN December 2013



PB Toxins Endothelium Activation

Evidences in Uremia to promote Aging

Human umbilical vein endothelial cells (HUVEC) incubated with uremic serum **Experimental Gerontology August 2014**

Uremic serum increased ROS

Uremic serum increased NF-κB

uremic serum induce apoptosis

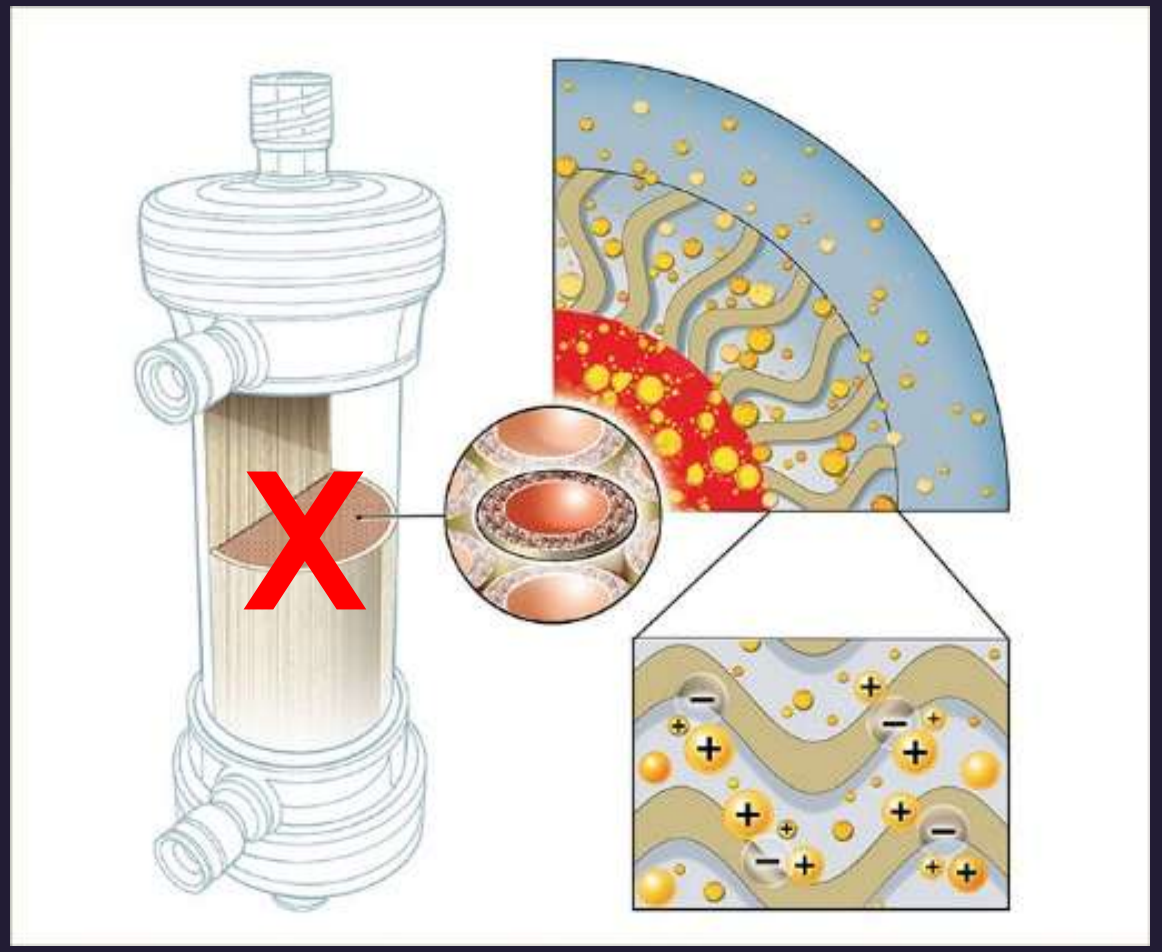
In Aging Cells

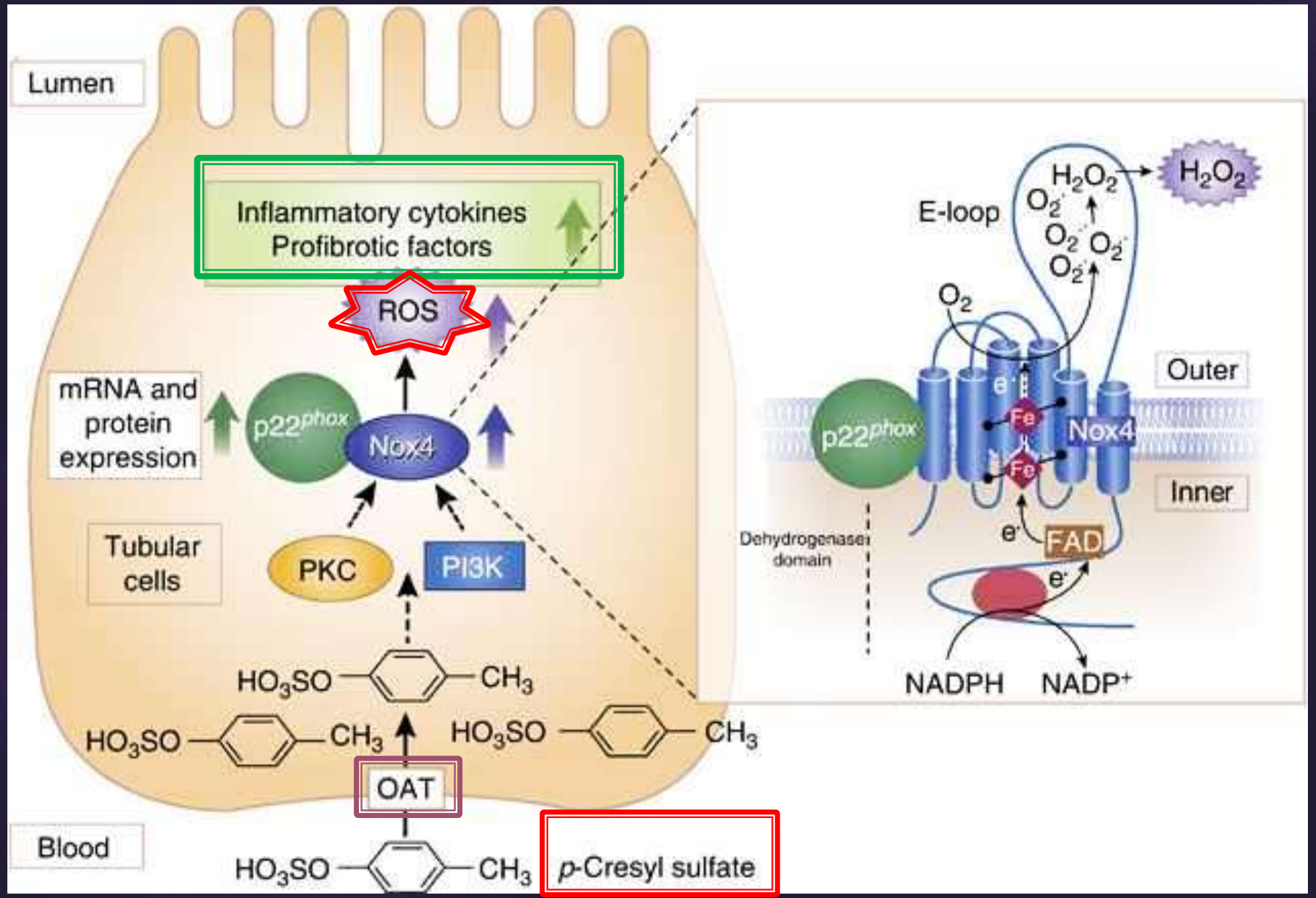
oxidative stress damage

senescent cells

PB Uremic Toxins
Gut feeling and Retention in uremics the marker of inadequate HD therapy

The Protein Bound Uremic Toxins Removed By Tubules not By filtration !





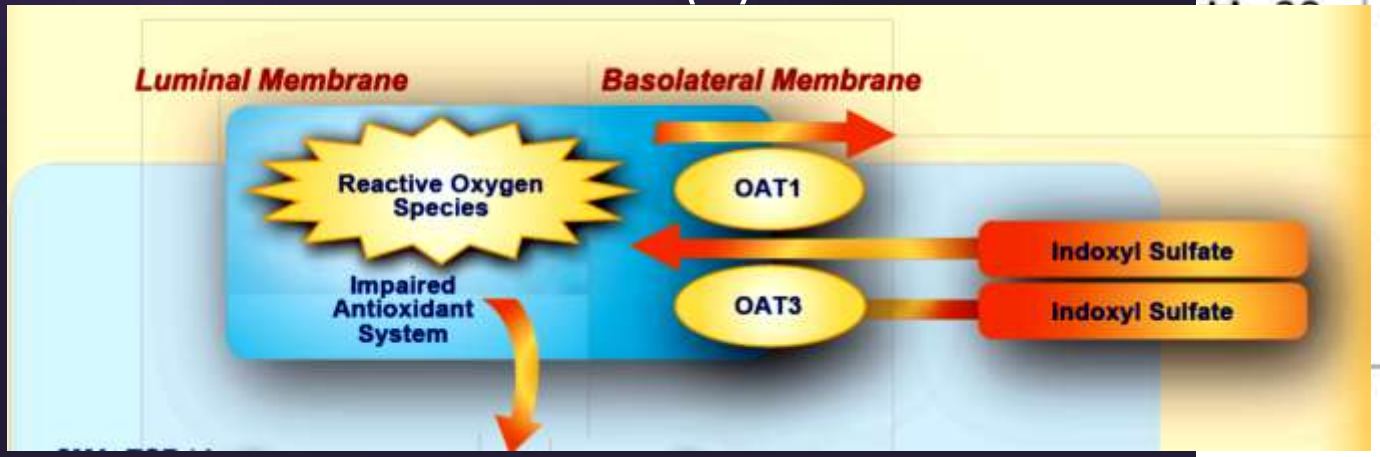
Kidney International (2013) 83

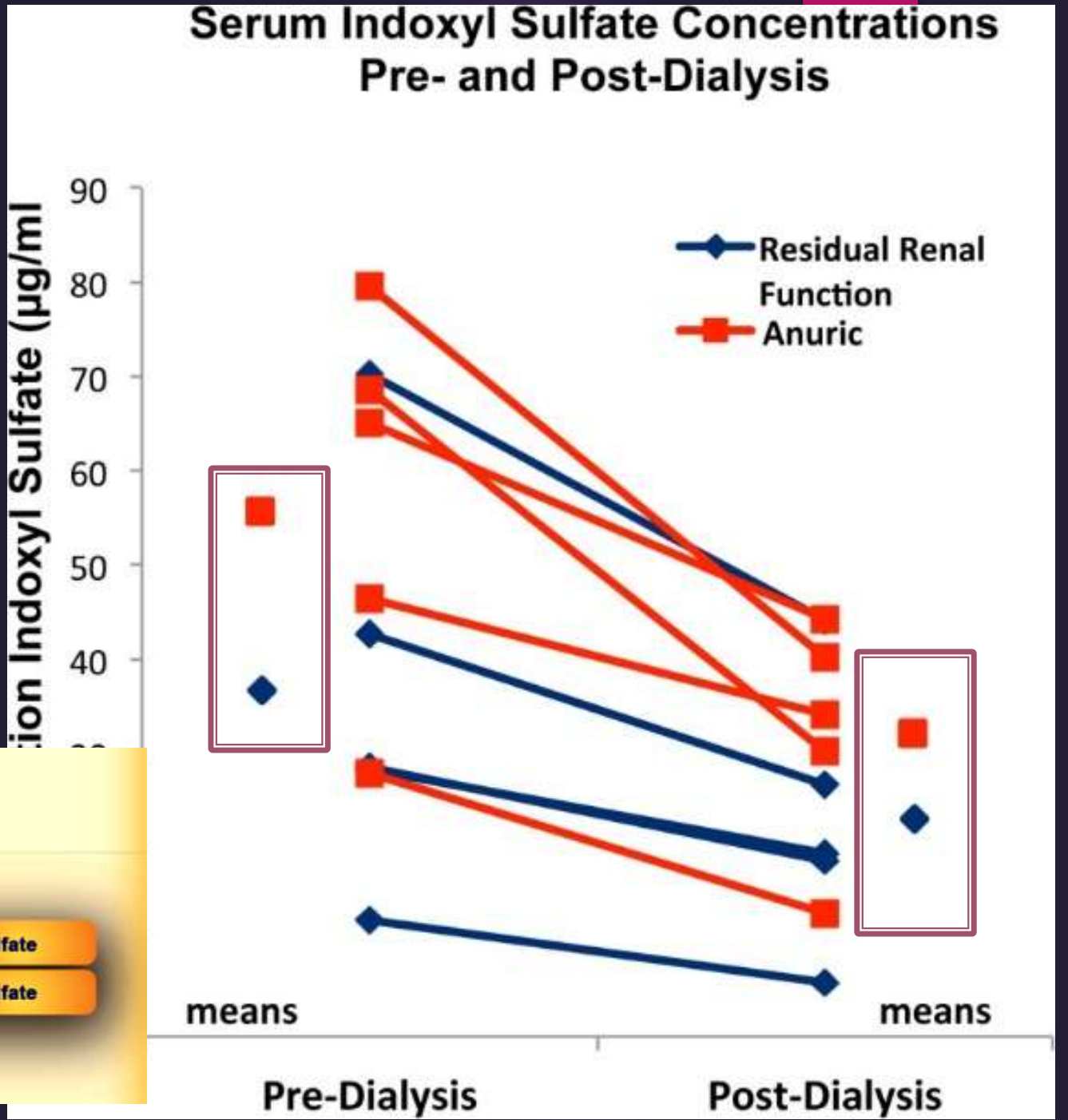
PB Toxins ↑ ROS Through NOX4 of NADPH oxidase

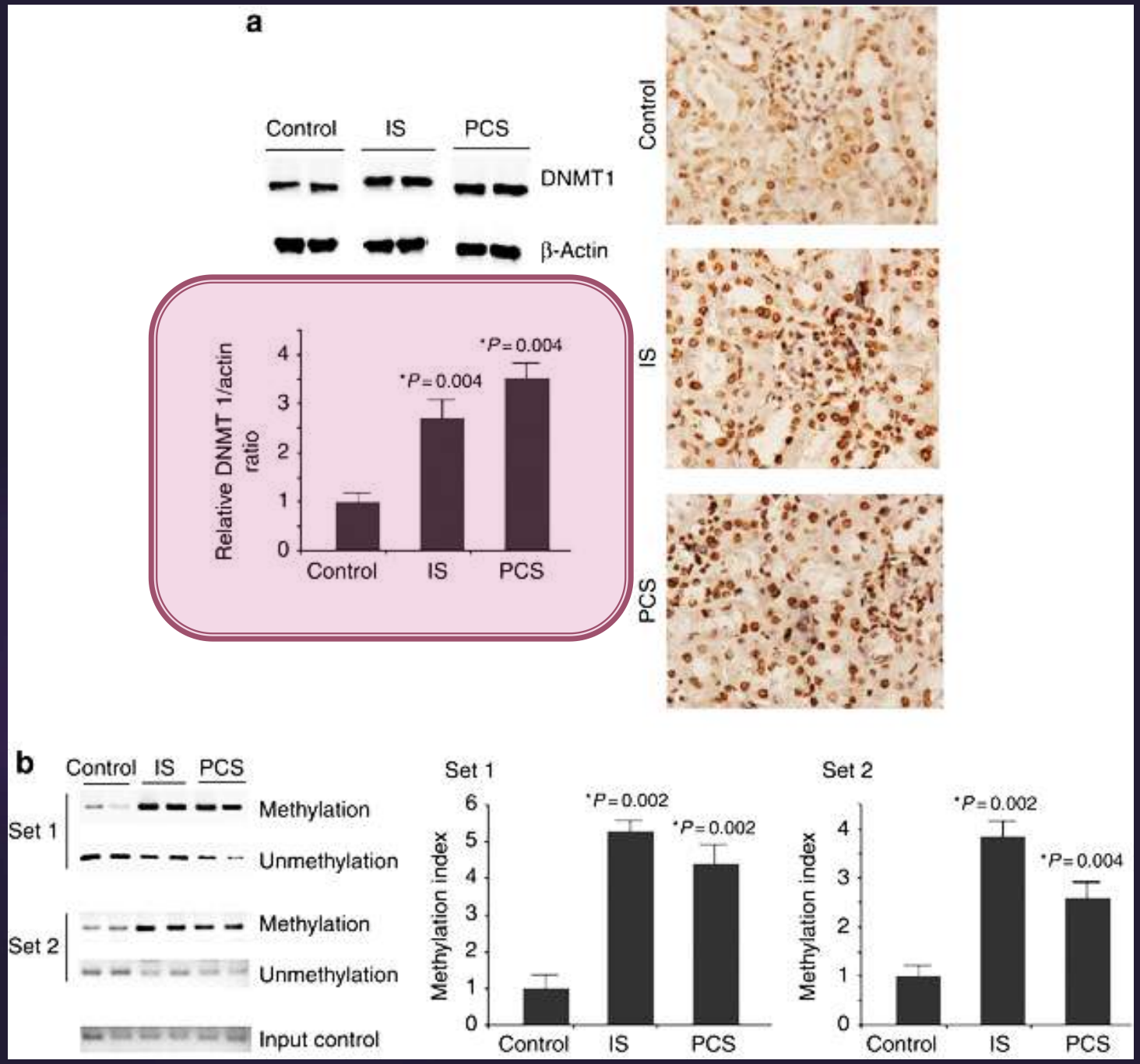
Indoxyl Sulfate

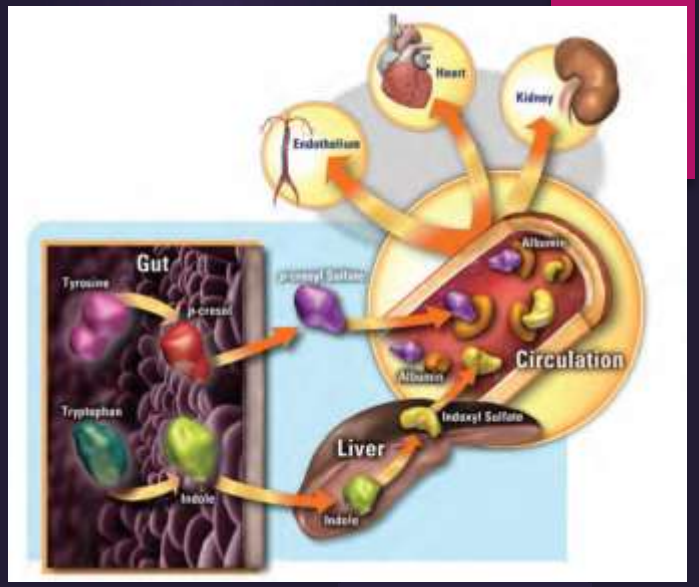
IS level is better in patients with
Residual Renal Functions
(Tubular Organiic Anion Transporter)
OATs

PLoS One v.10(3); 2015









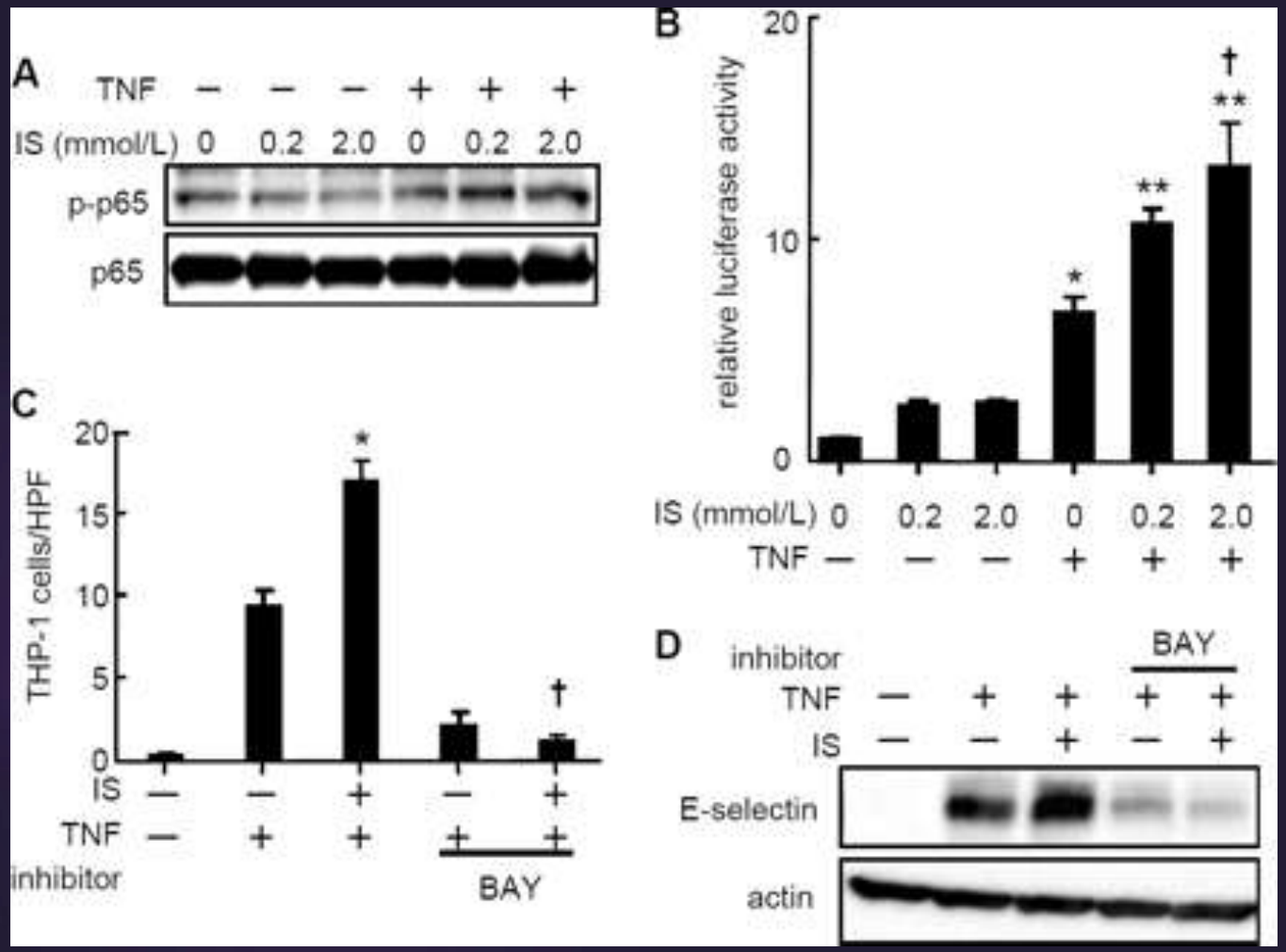
Indoxyl sulfate (IS)- and p-cresyl sulfate (PCS)-injected mice had increased DNA methyltransferase 1 (DNMT 1) expression and DNA hypermethylation of the Klotho gene

Kidney International (2012) 81, 640–650

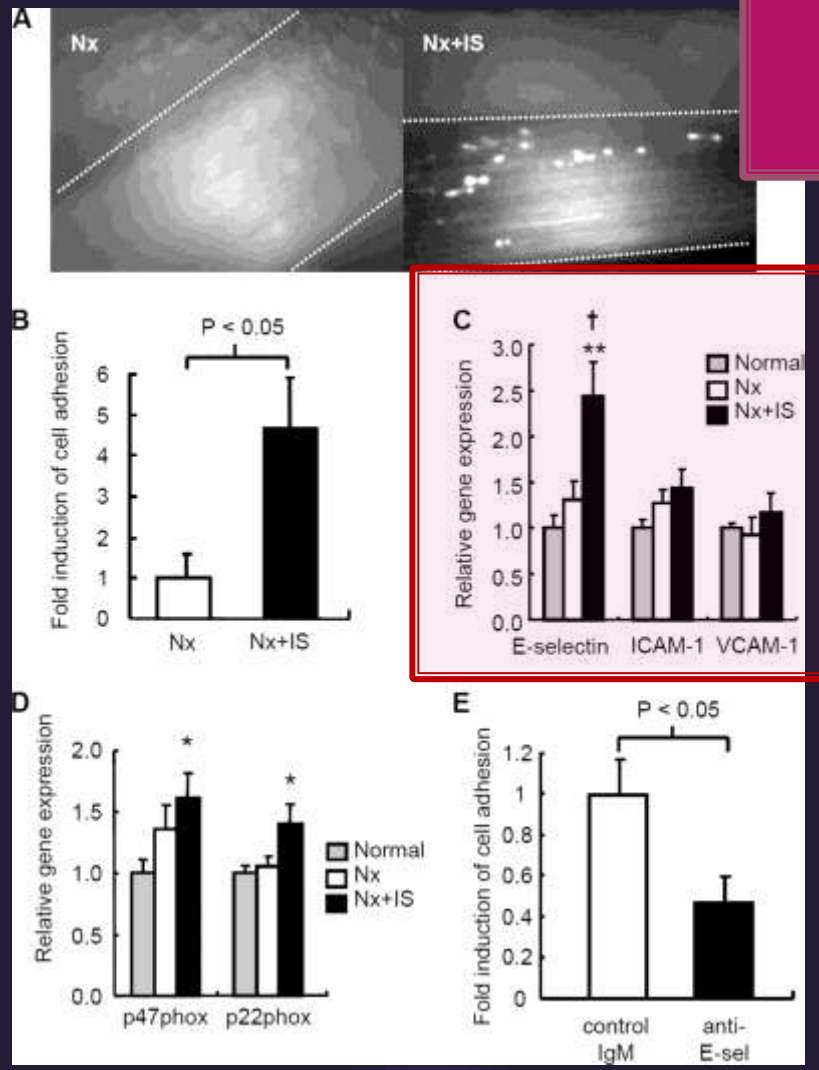
Indoxyl Sulfate Induces Leukocyte-Endothelial Interactions through Up-regulation of E-selectin , ICAM , VCAM and TNF a

Journal of Biological Chemistry ,Dec 2010

Nx: only water
Nx IS : IS

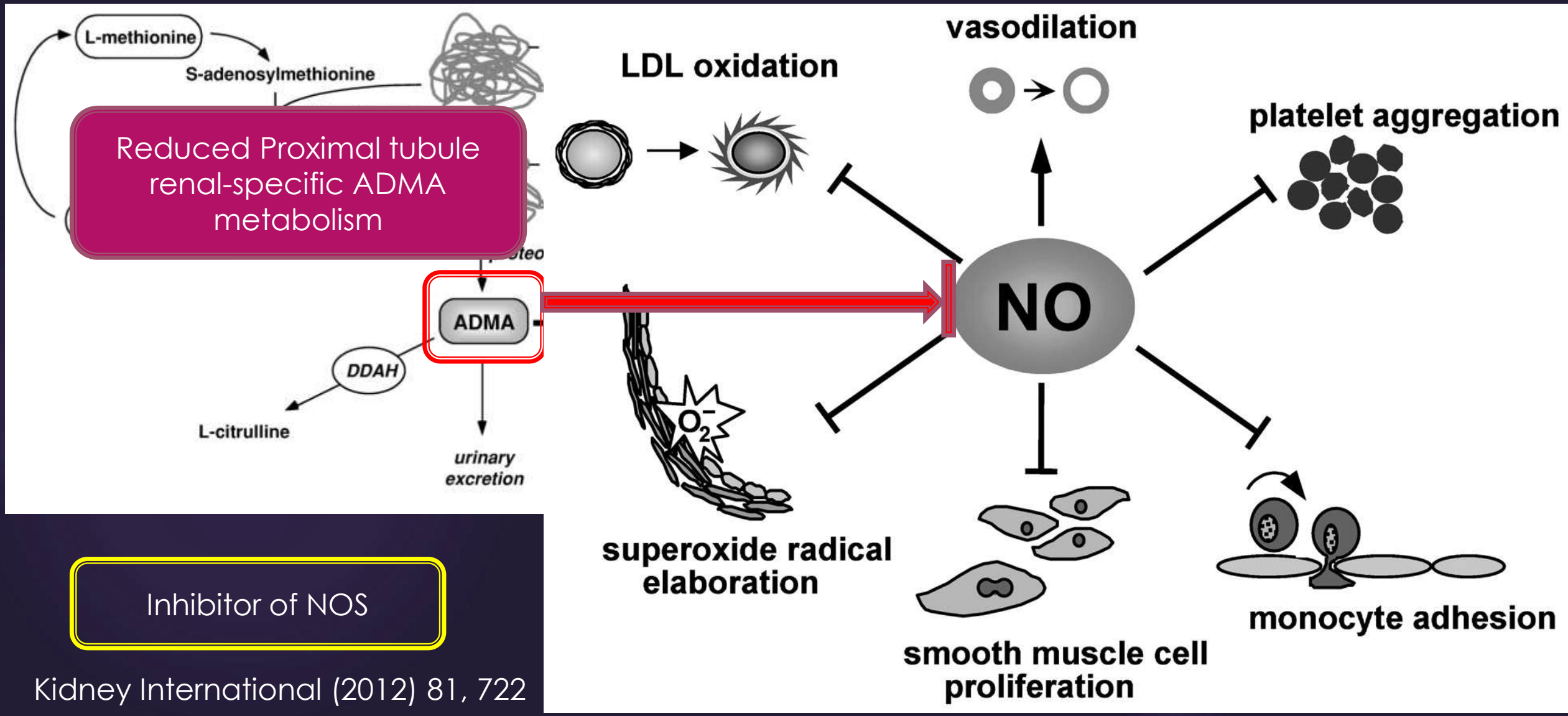


Effects of indoxyl sulfate on the TNF-a-induced NF-κB pathway



ADMA : PB Toxins Retention

J Am Soc Nephrol. 2015 Apr



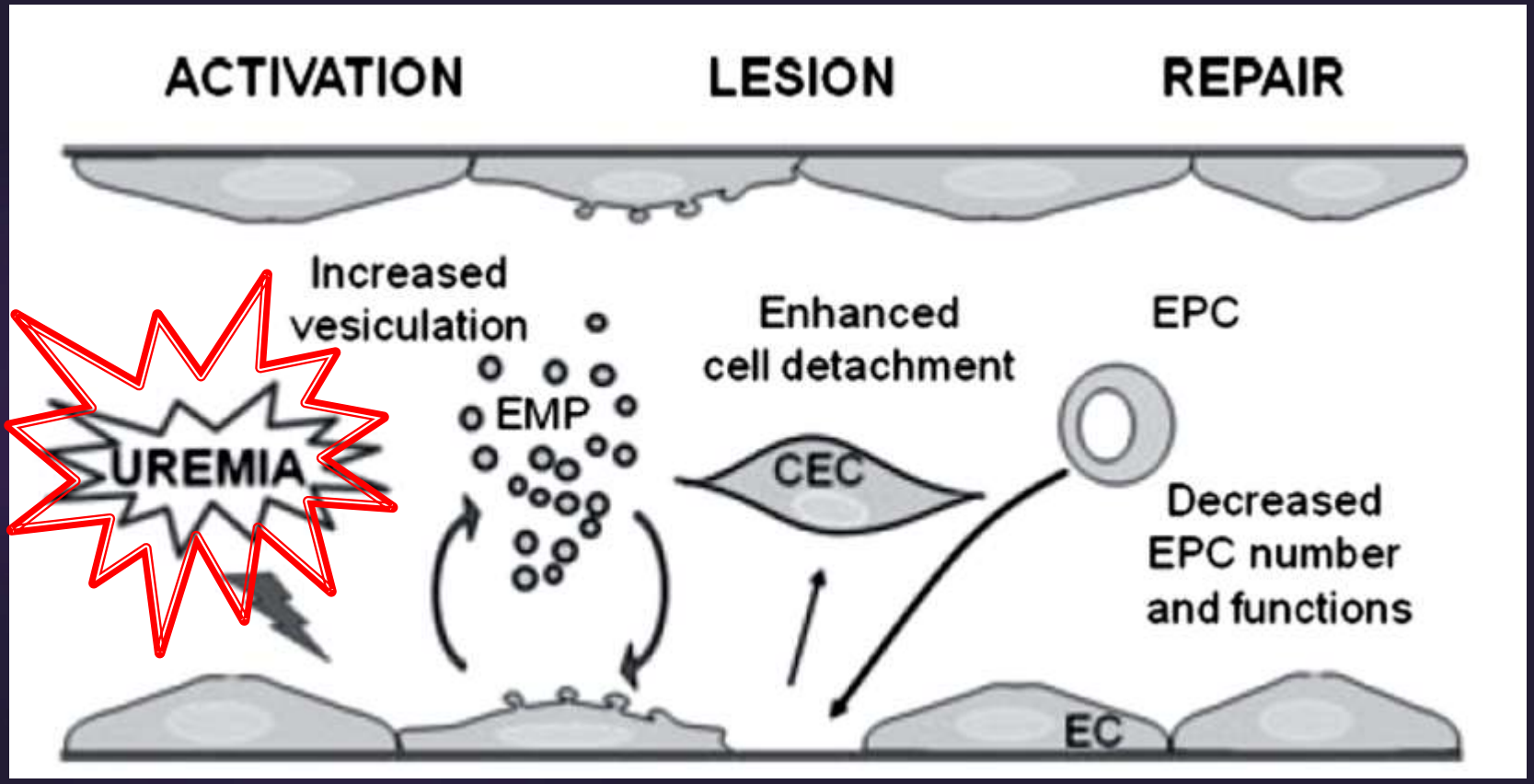
Inhibitor of NOS

Kidney International (2012) 81, 722

Circulating Endothelial Cells (CES)

BioMed Research International Volume 2014

- CEC counts are increased in diseases associated with a high degree of endothelial cell activation and/or injury



(1) Uremia induces the release of endothelial microparticles (EMP)

(2) increase in circulating endothelial cells (CEC)

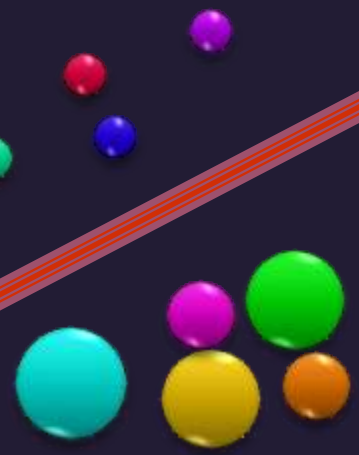
(3) uremia impairs the survival of endothelial progenitor cells (EPC)

combination of different surface antigens such as CD146, CD45, and CD31 to detect the endothelial cells

Removal of PB uremic Toxins the philosophy of therapy

Future HD
Adequacy marker

HD remove only free form < 5%



PB Toxins

- Remove by adsorption
- Membrane Block
- (fouling)

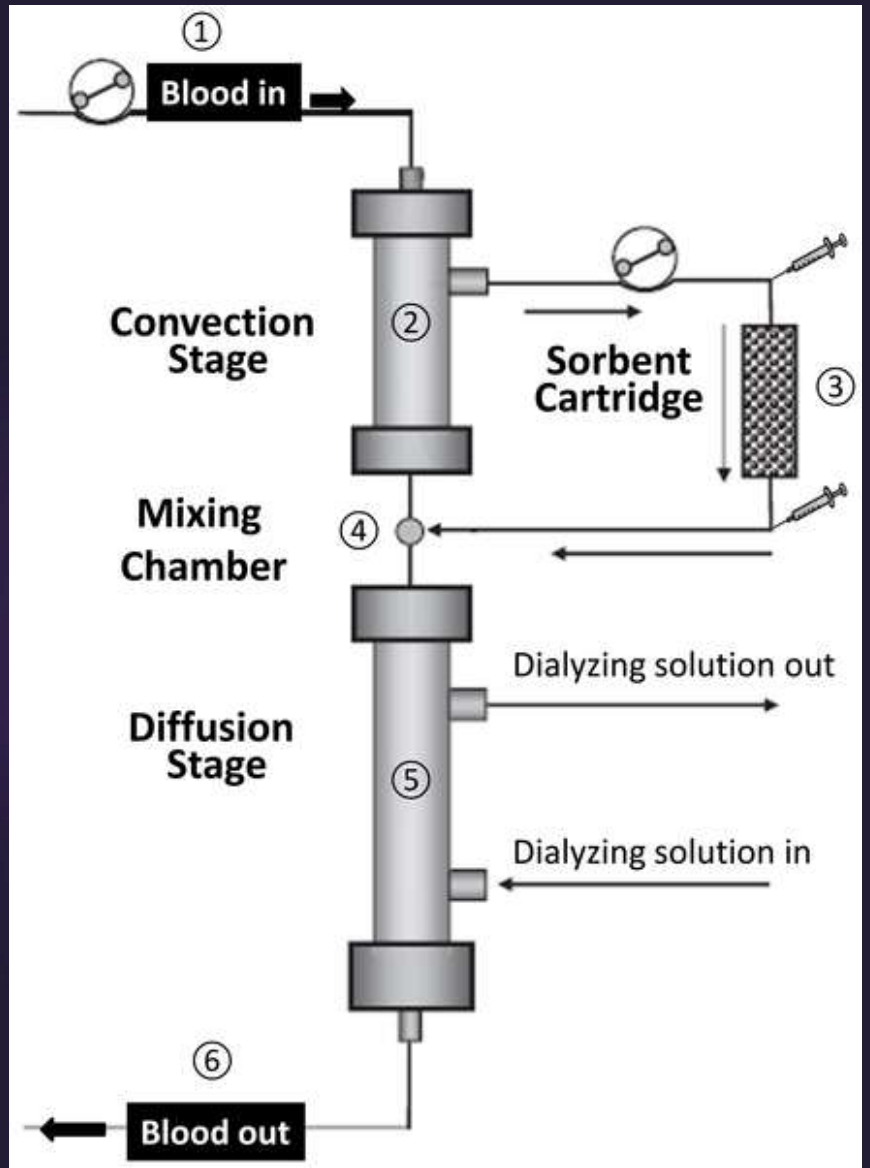
Tubular Function

TRT

- BAK
- Implantable devices

Towards Adsorption Techniques

plosone.org April 2014 | Volume 9

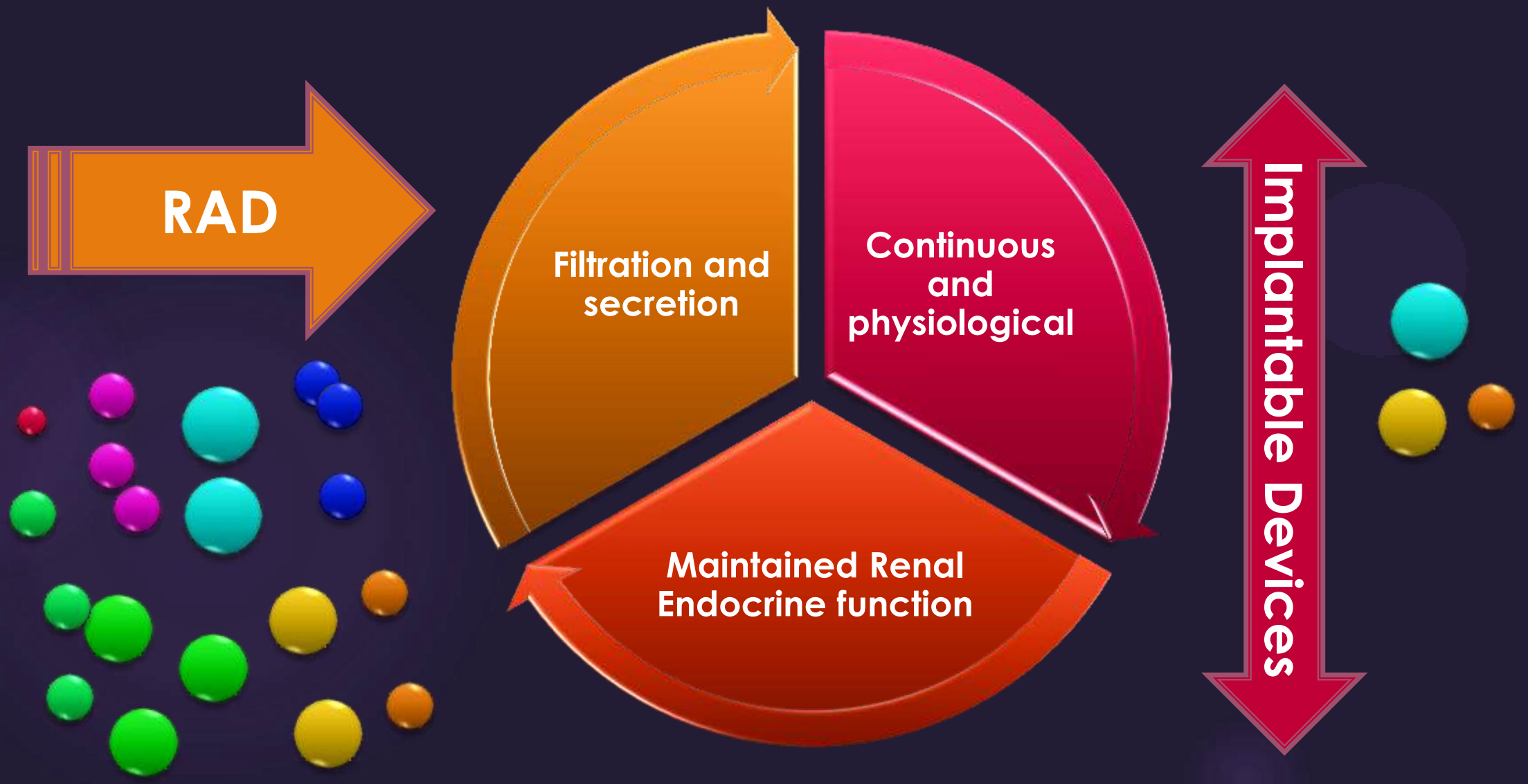


Albumin sieving coefficient of 0.02.

the use of an adsorber in combination with dialysis membranes may be a new therapeutic option to increase the removal rate of these uremic toxins

Artificial Organs Volume 37, Issue 4, pages 409–416, April 2013

The Future Towards



BAK : The theory and the promise!

- placing renal tubule cells, primary cells or cell lines established from various species, on the inner surface of hollow fibers.

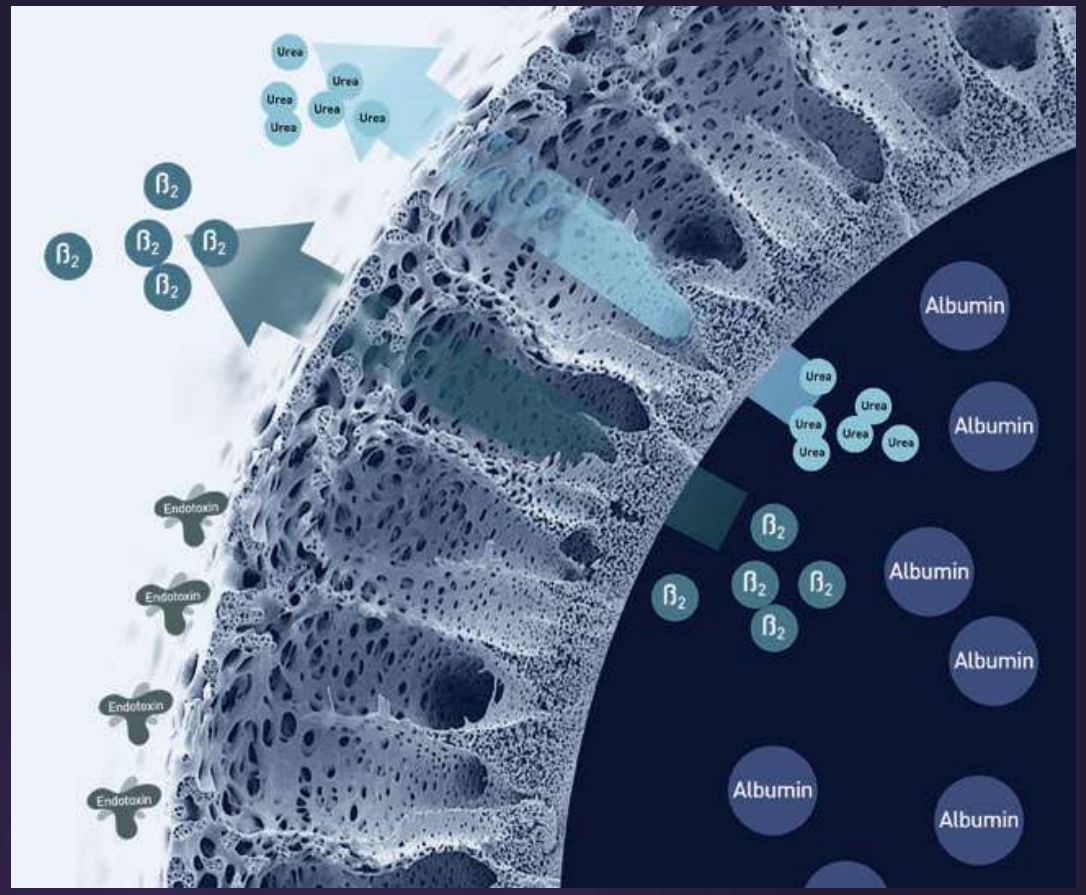
BAK

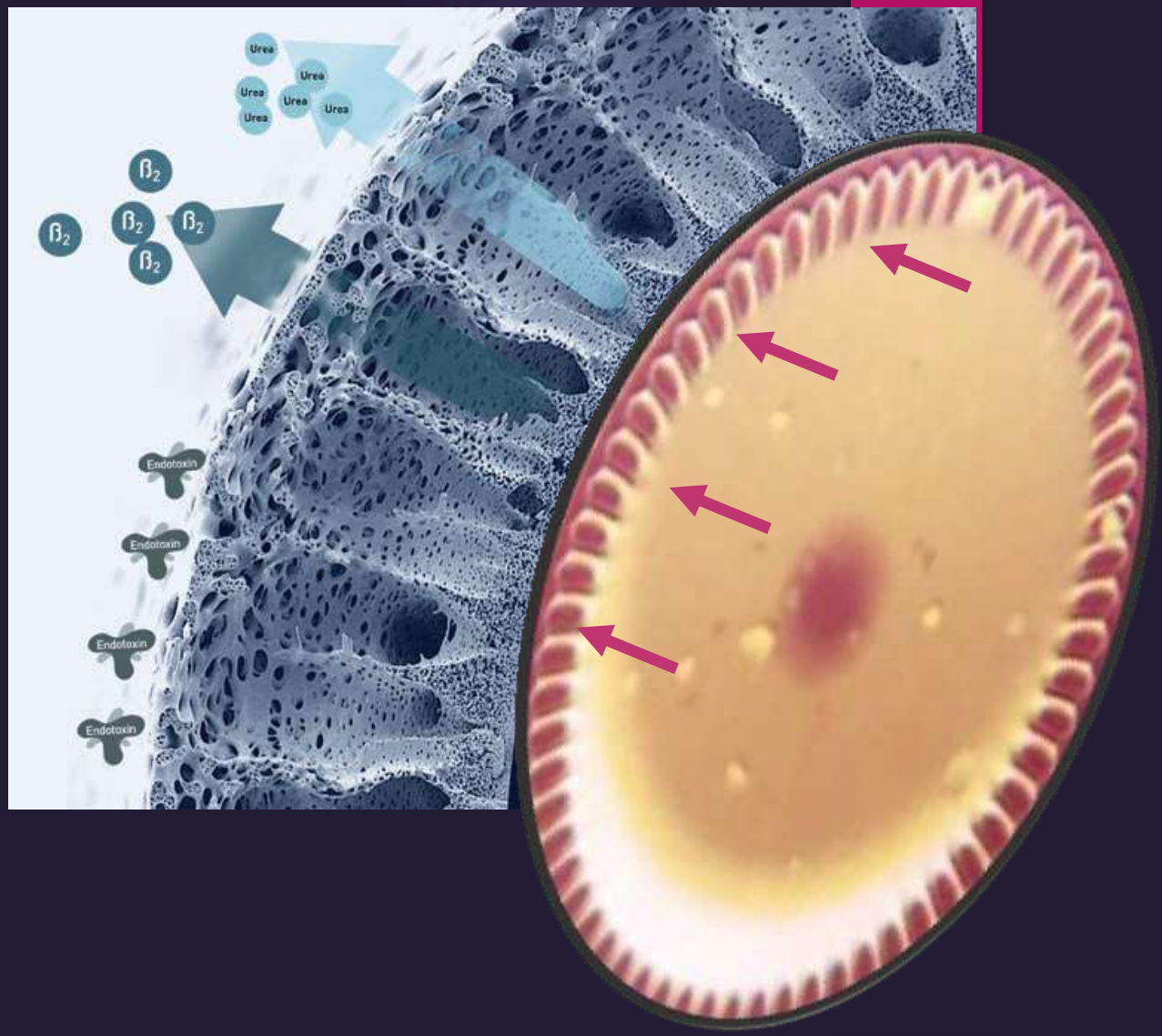
SECRETION

REABSORPTION

ENDOCRINE

METABOLIC

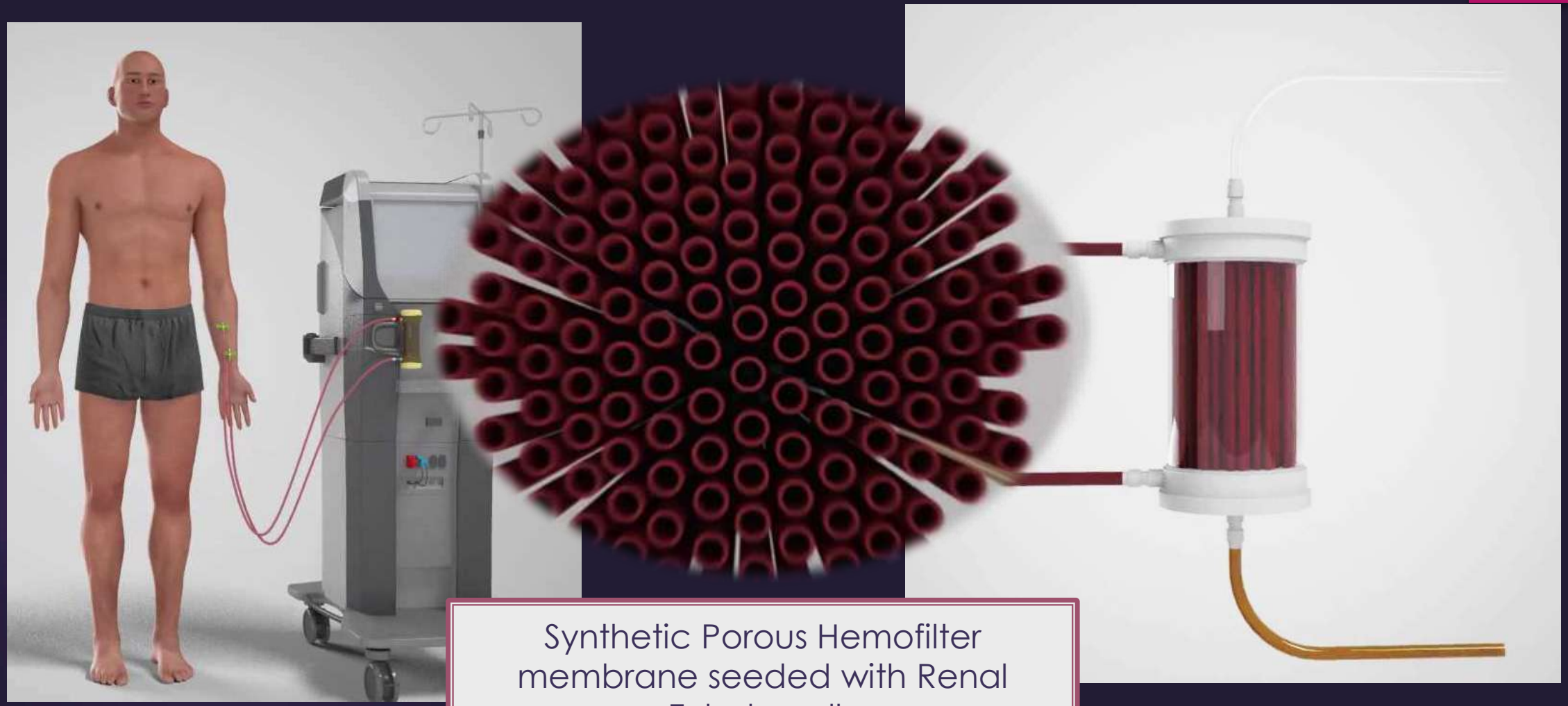




Filtration

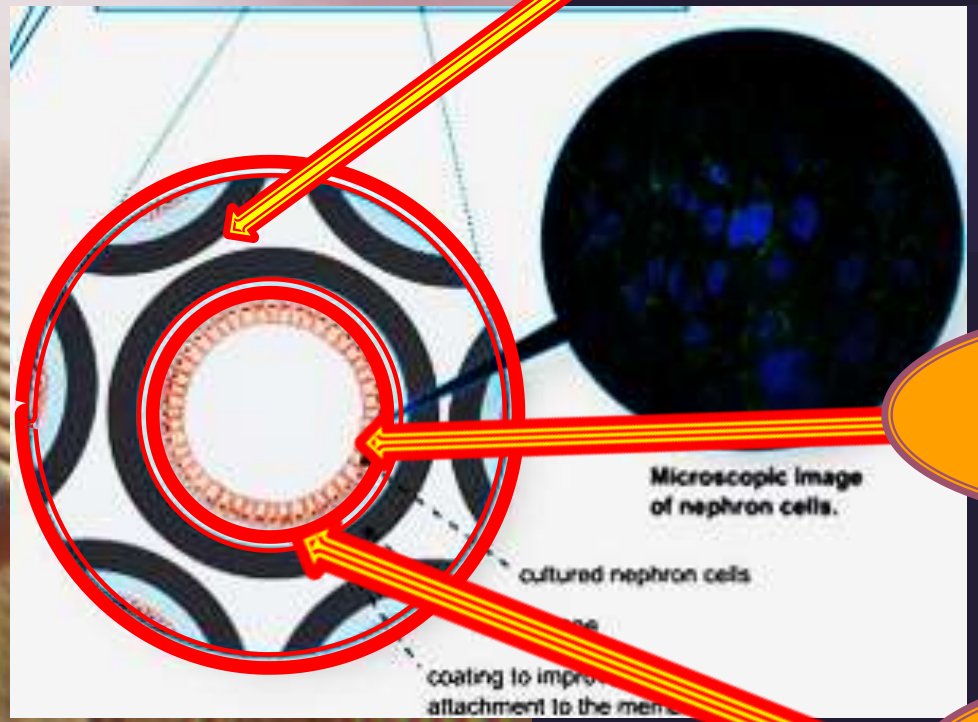
RAD : Filtration Secretion

BAK : The theory and the promise!



Synthetic Porous Hemofilter membrane seeded with Renal Tubule cells

PB Toxins



Hemofilter Membrane

Monolayer With Tight Junctions

Surface coating

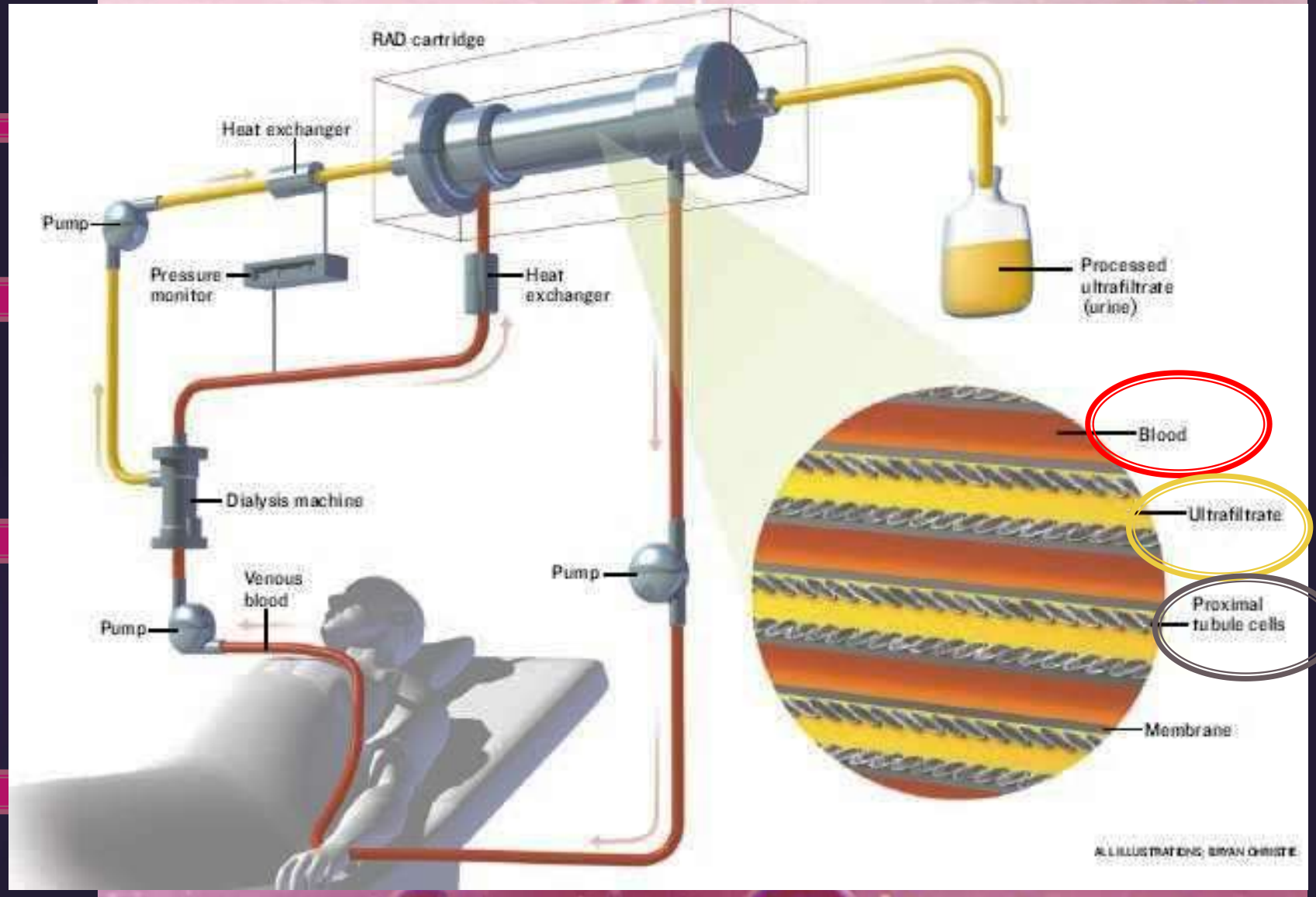
BAK : The theory and the promise

Blood Flow

Outer membrane layer 1 um pores

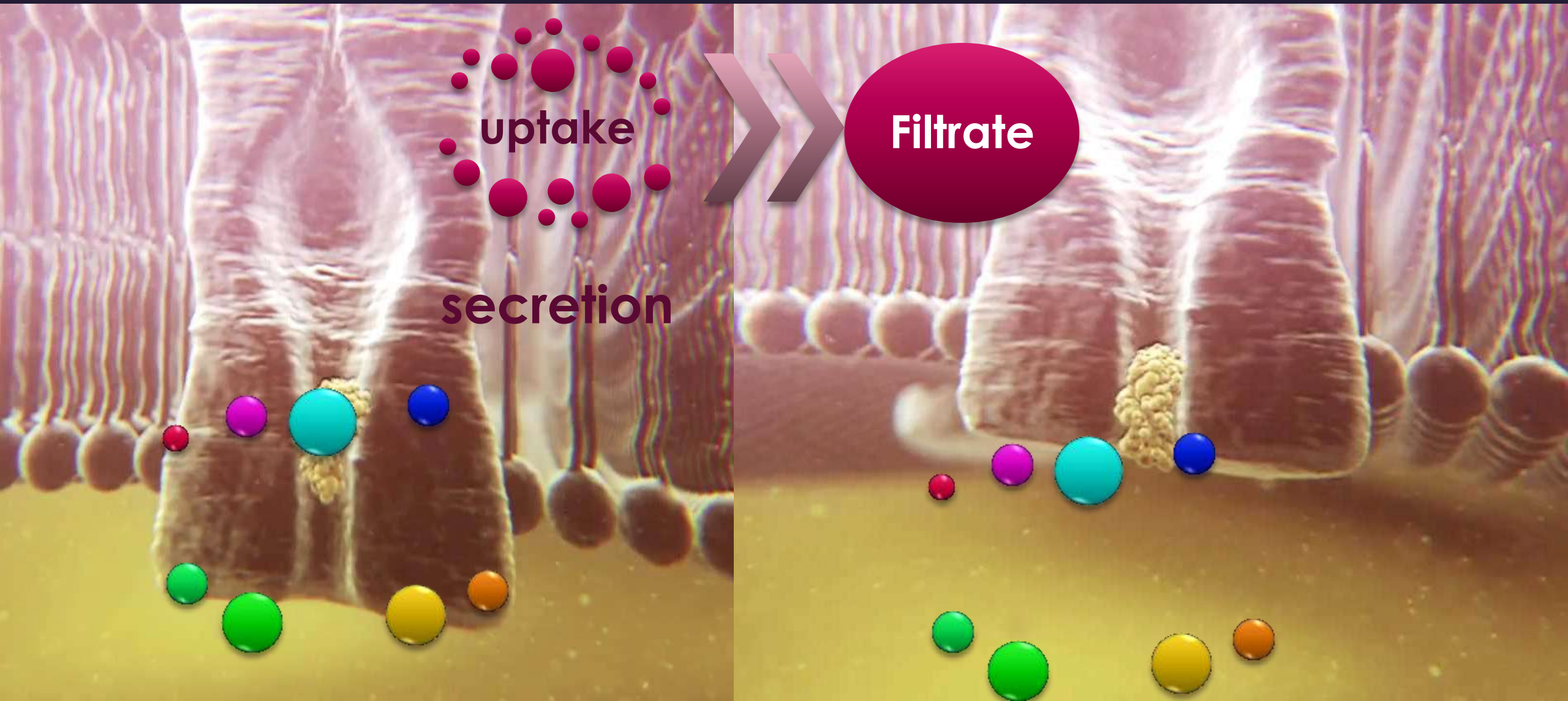
Filtrate flow for Reabsorption

Outer layer clogging by proteins and cells

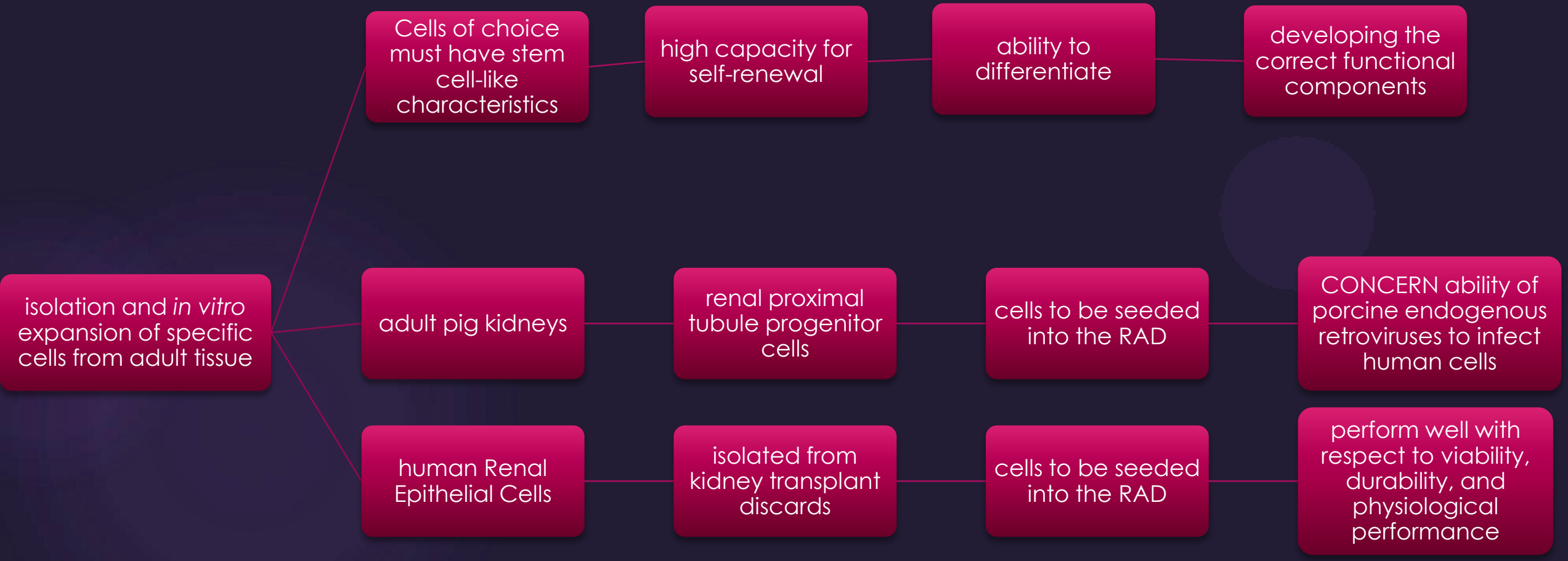


International Journal of Tissue Engineering Volume 2013

BAK : The theory and the promise



Challenge: tissue source and supply



Humes etal Kidney Int. 1999; 55: 2502–2514

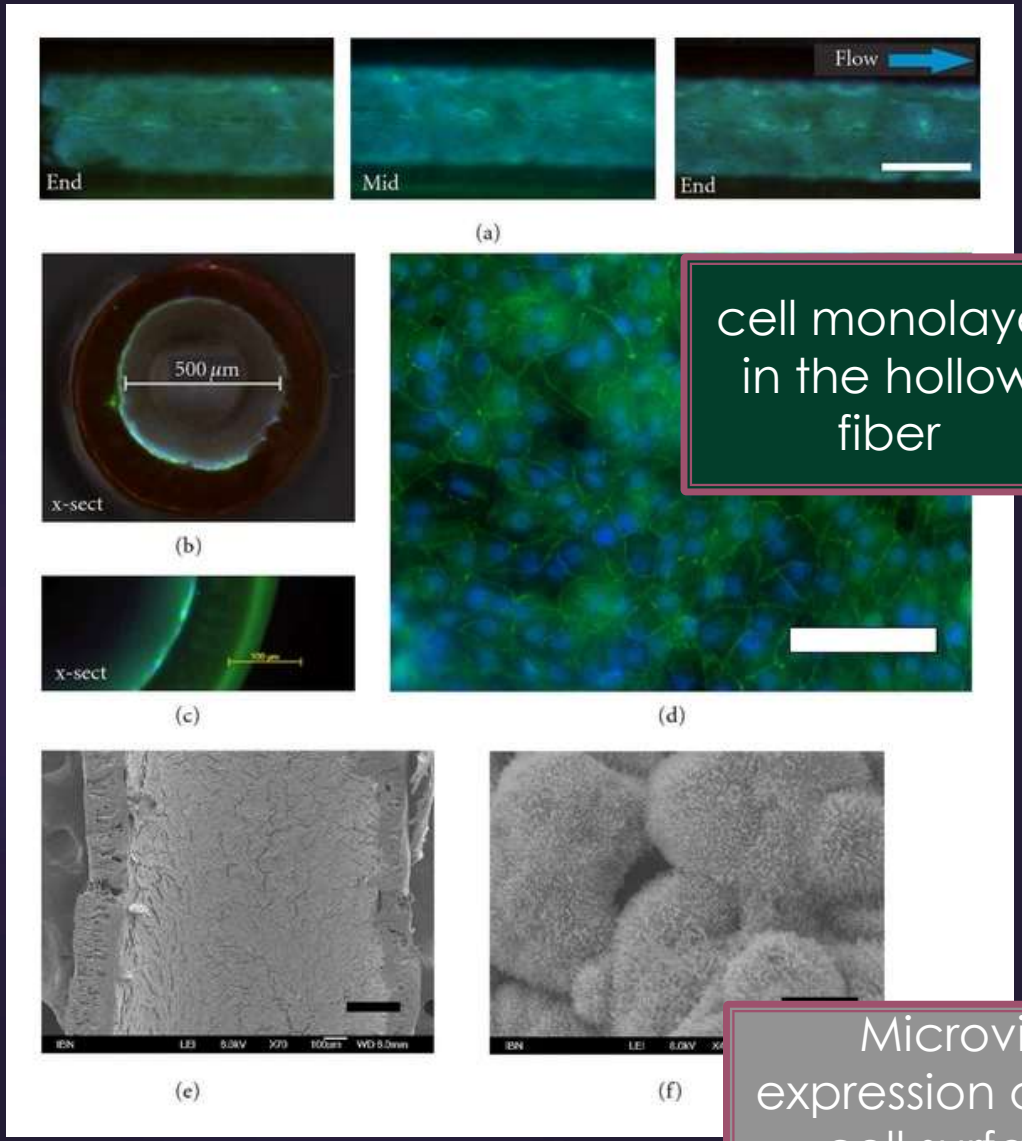
Paradis etal ,Science. 1999; 285: 1236

Buffington etal, Cell Med. 2012; 4:

Westover etal ,J Tissue Eng Regen Med. 2012; 6

BAK requirements

International Journal of Tissue Engineering Volume 2013



cell monolayer in the hollow fiber

Microvilli expression on the cell surface

Renal Epithelial Cells

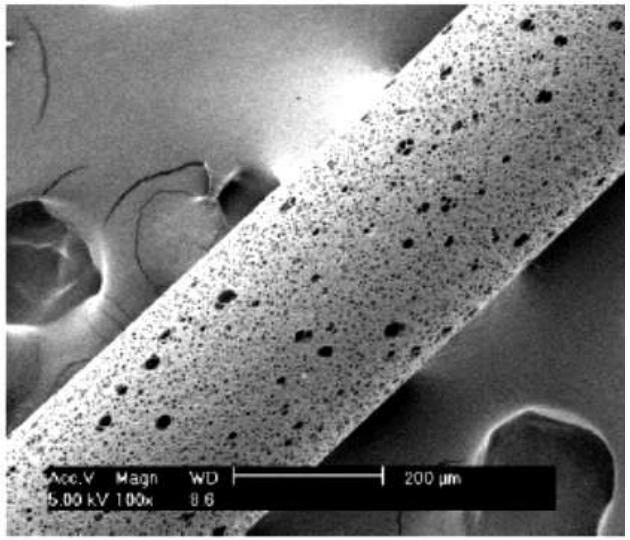
Homogenous monolayer

Porous membrane

- **PS**
- **PES**
- **PAES**

Coating to the membrane for Cell Attachment

- Pronectin.
- laminin .
- Bovine collagen type IV .
- Fibrin



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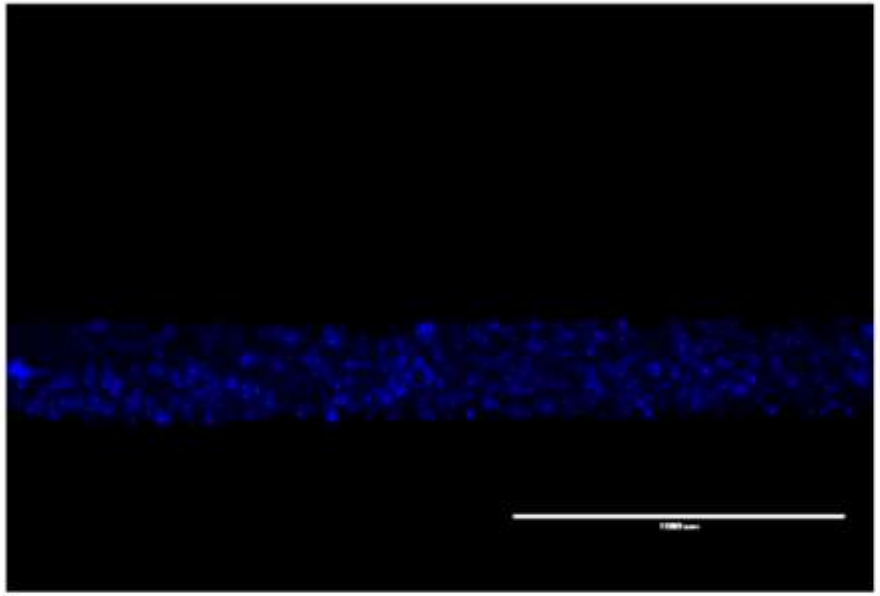

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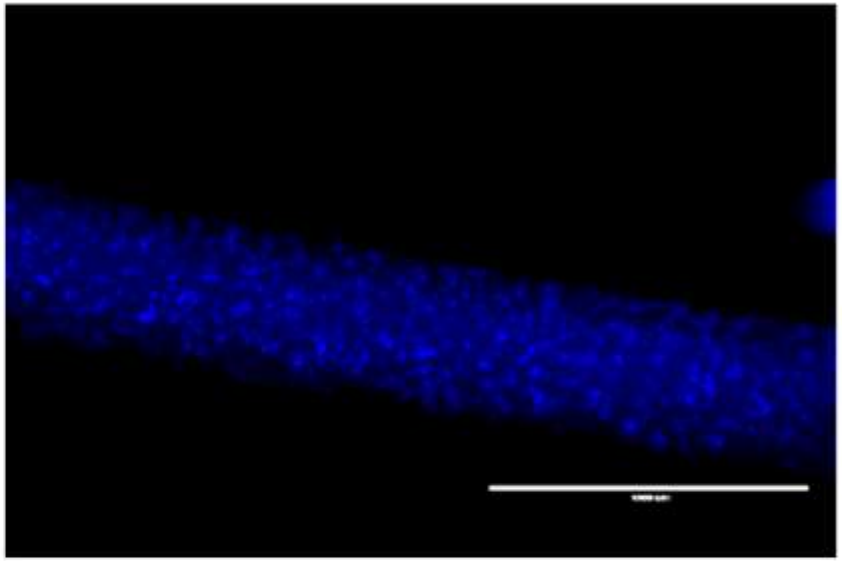
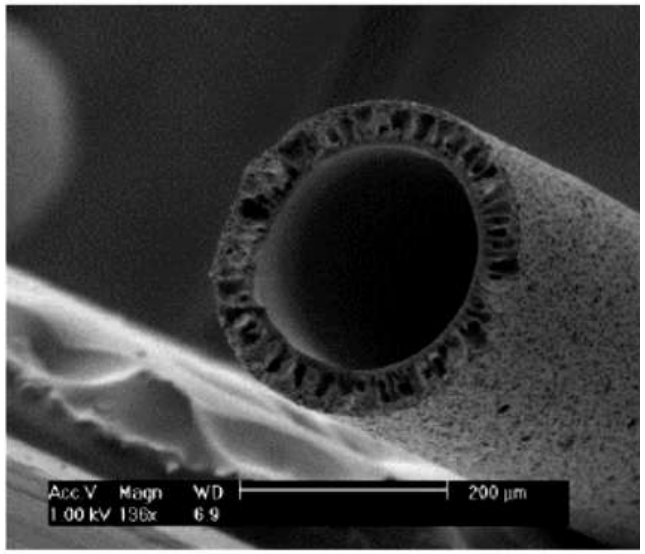
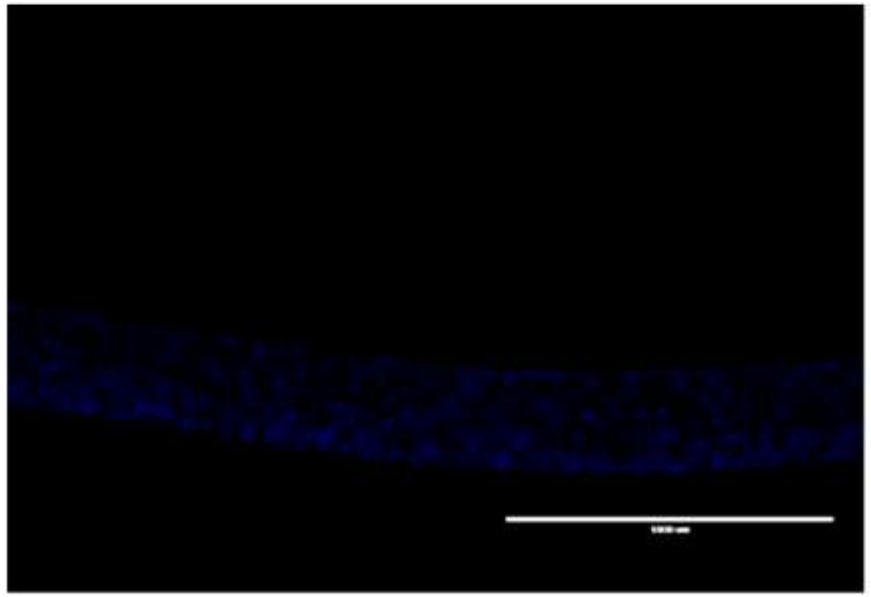
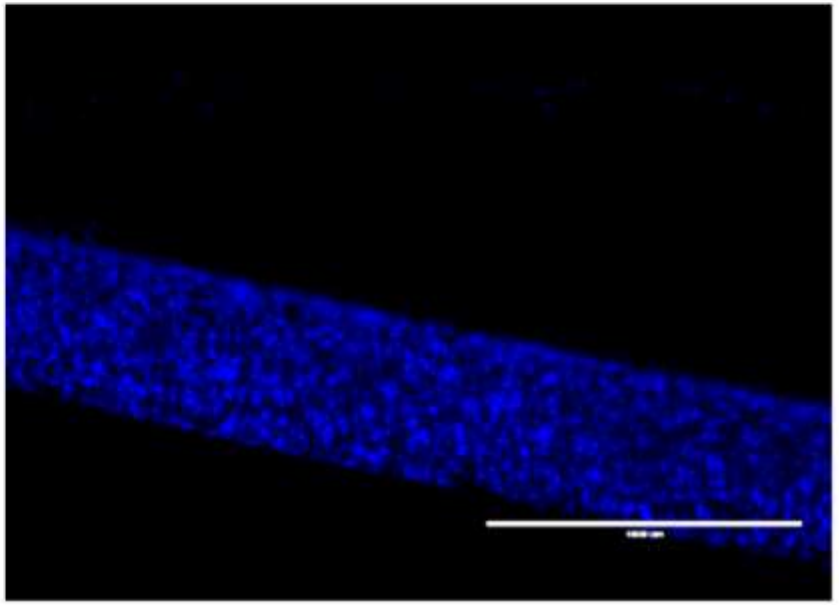


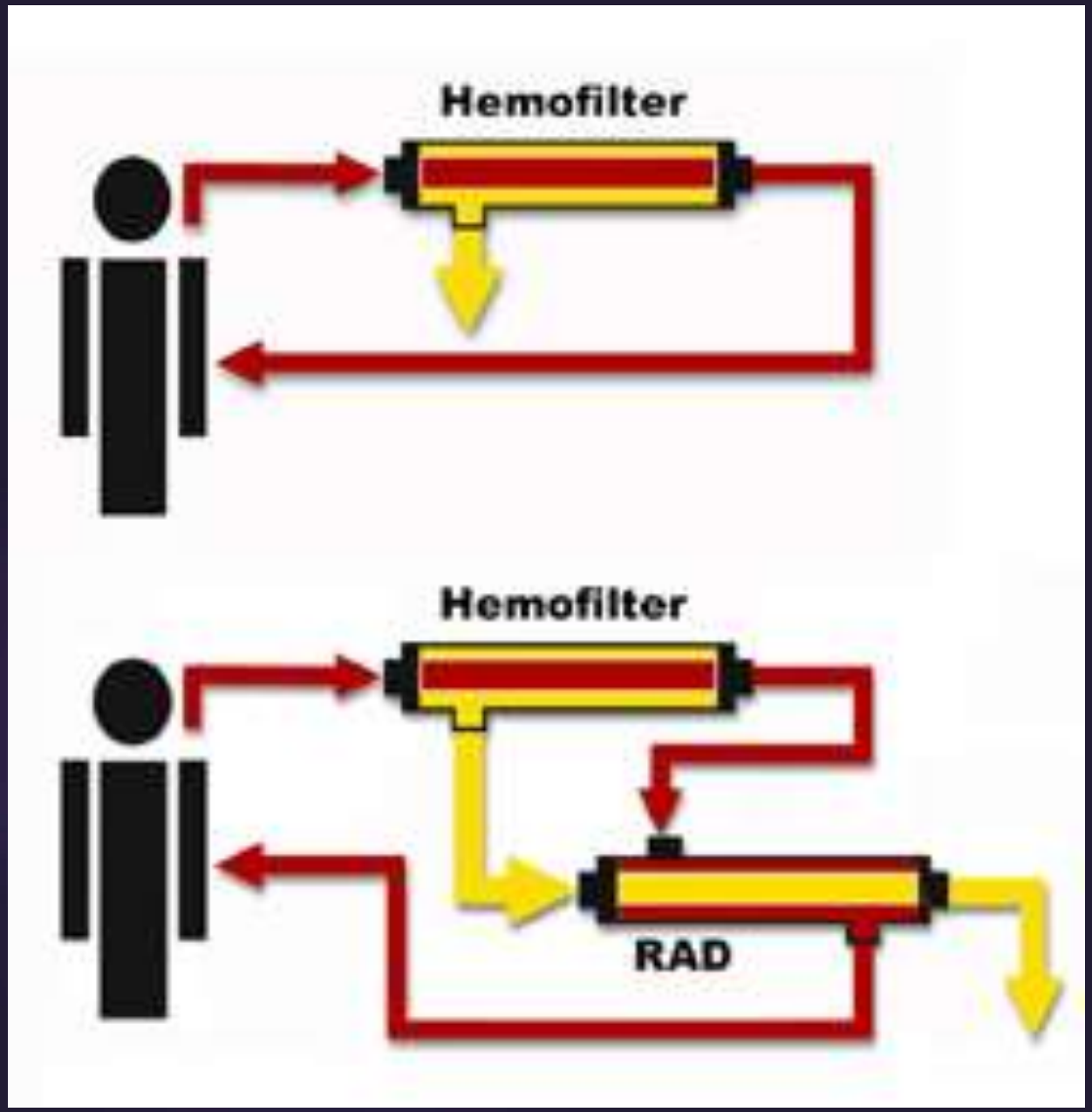




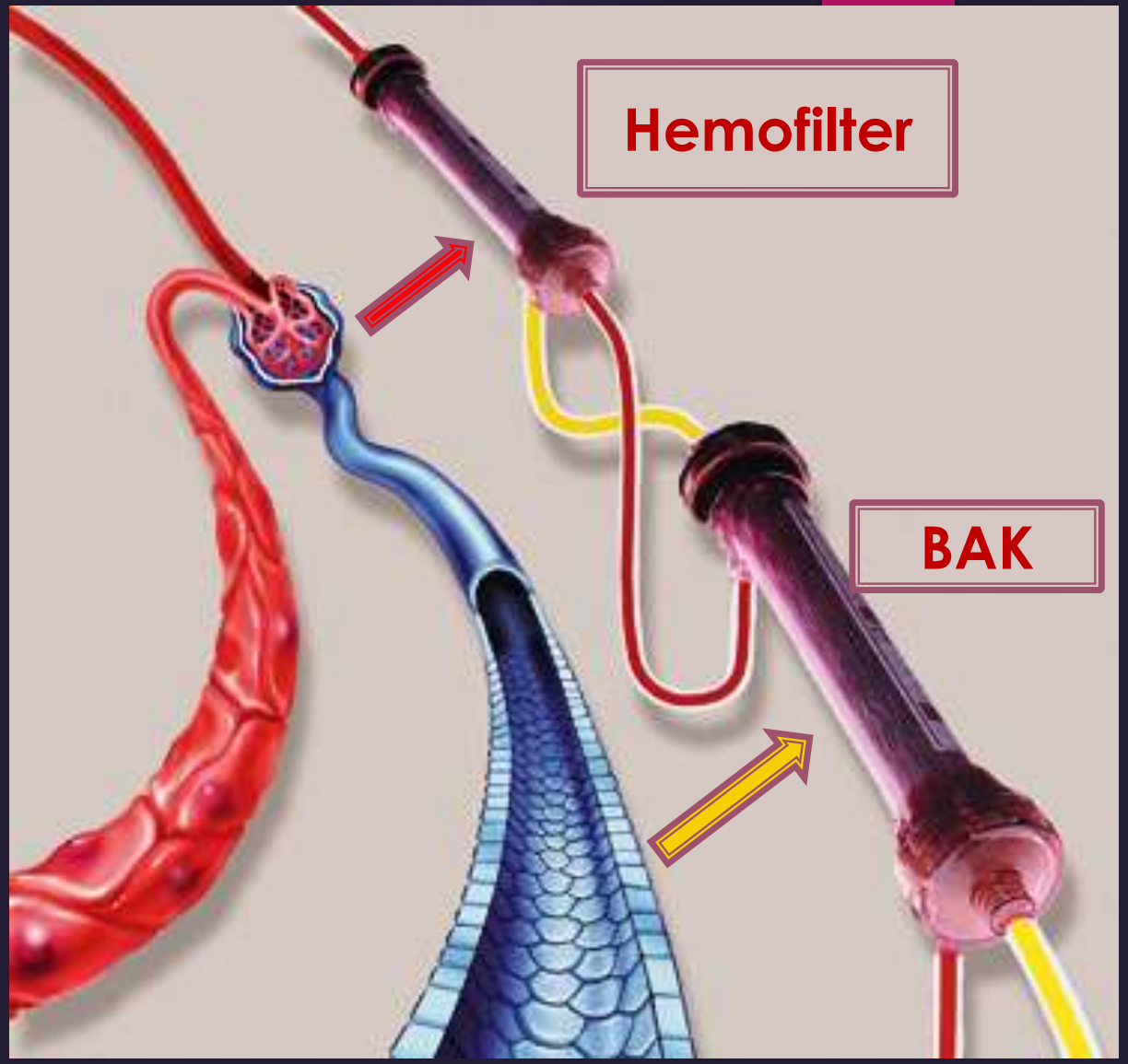
c



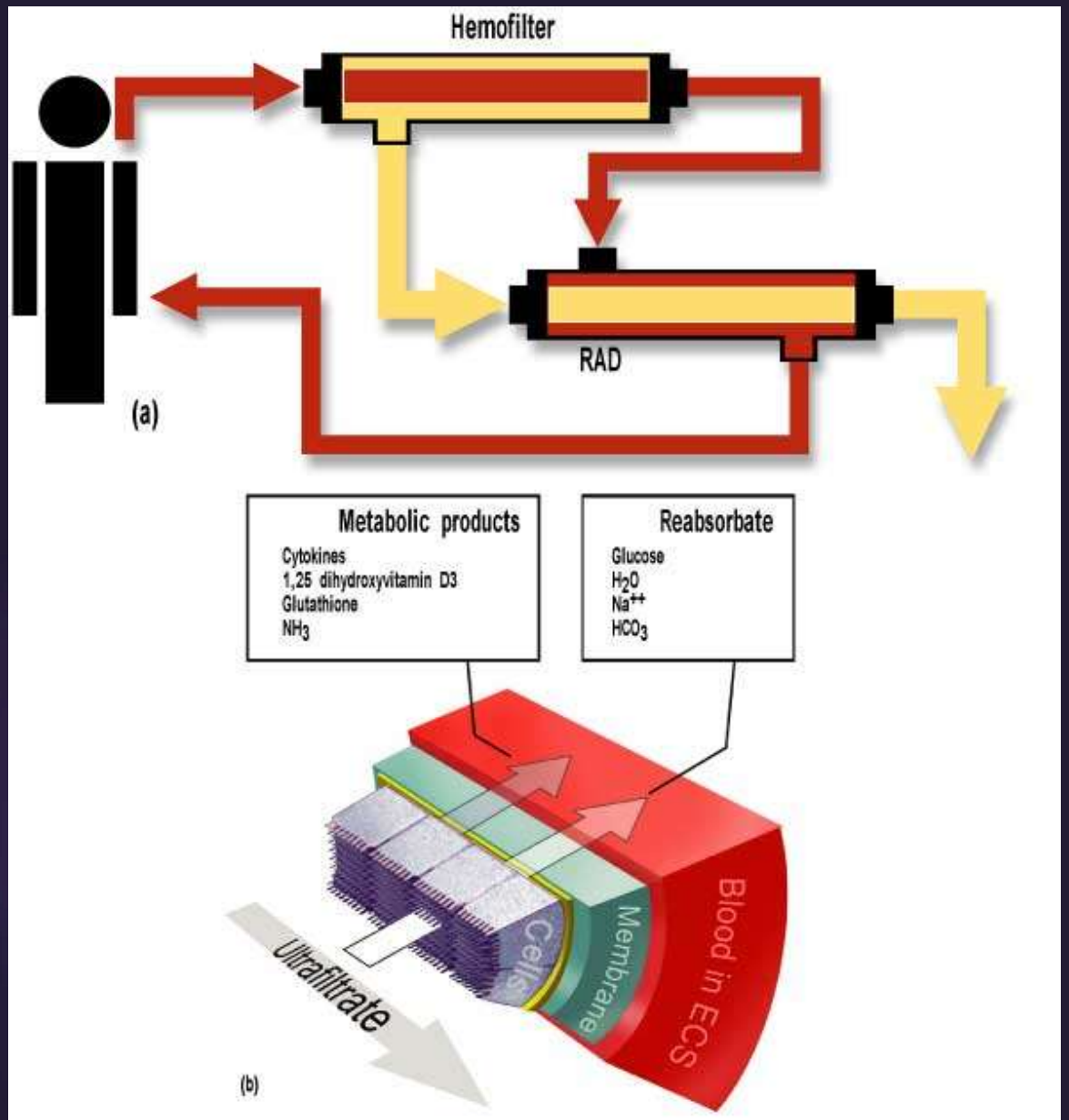
DAPI fluorescent staining for the nuclei of cells attached to the surface of HCO



Conventional treatment vs. BAK



Natural kidney function vs. BAK

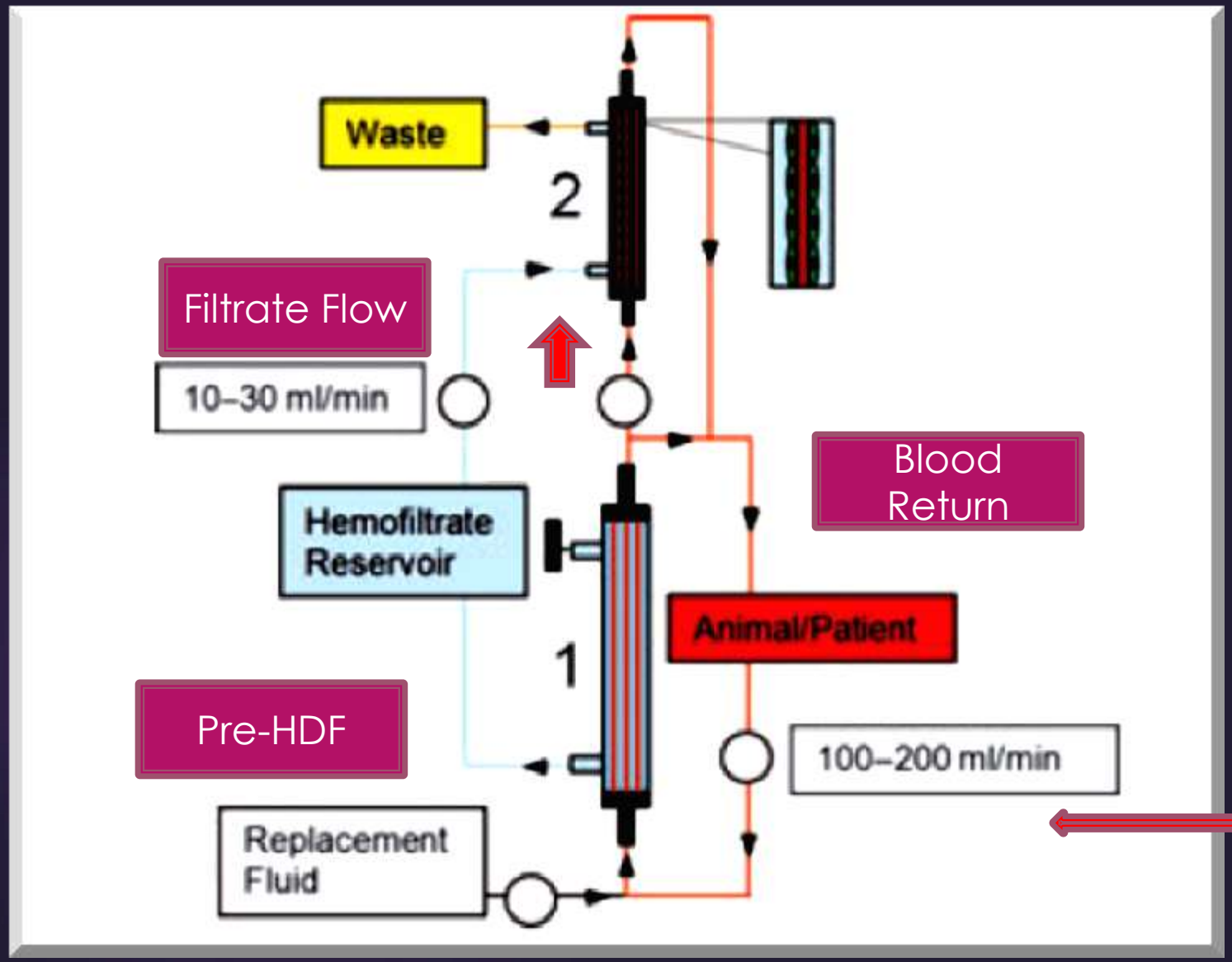


1. This RAD, developed at the University of Michigan and Nephros Therapeutics, improves a conventional kidney dialysis machine's role by returning useful substances, typically removed by the dialysis machine, to the bloodstream (a). For filtering purposes, the RAD makes use of reabsorbate and metabolic material (b).

The renal tubule cell assist device (RAD) is an extracorporeal device with a standard hemofiltration cartridge covered by nonautologous human renal tubular cells along the inner surface

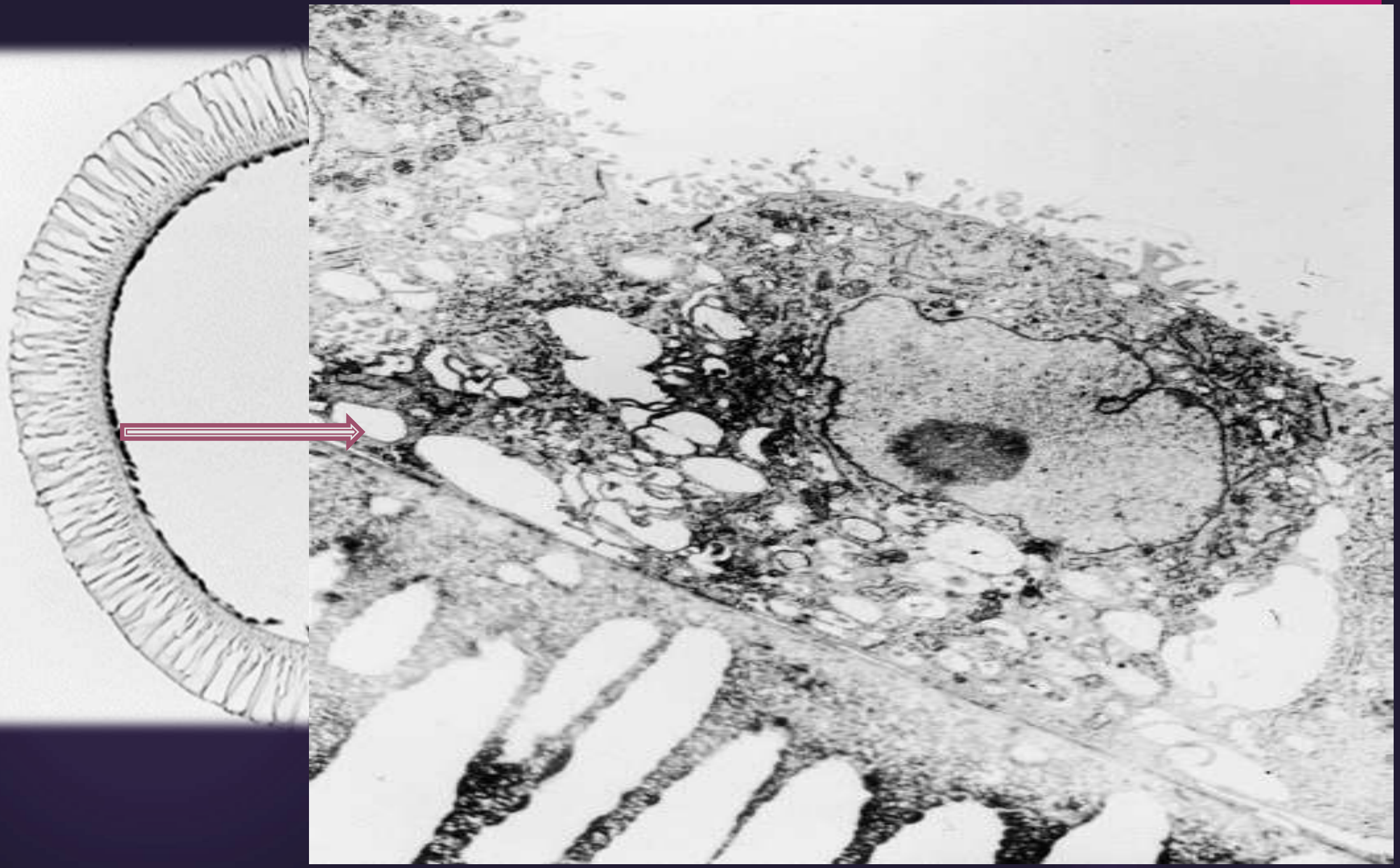
Journal of the American Society of Nephrology,2008 19, 1034

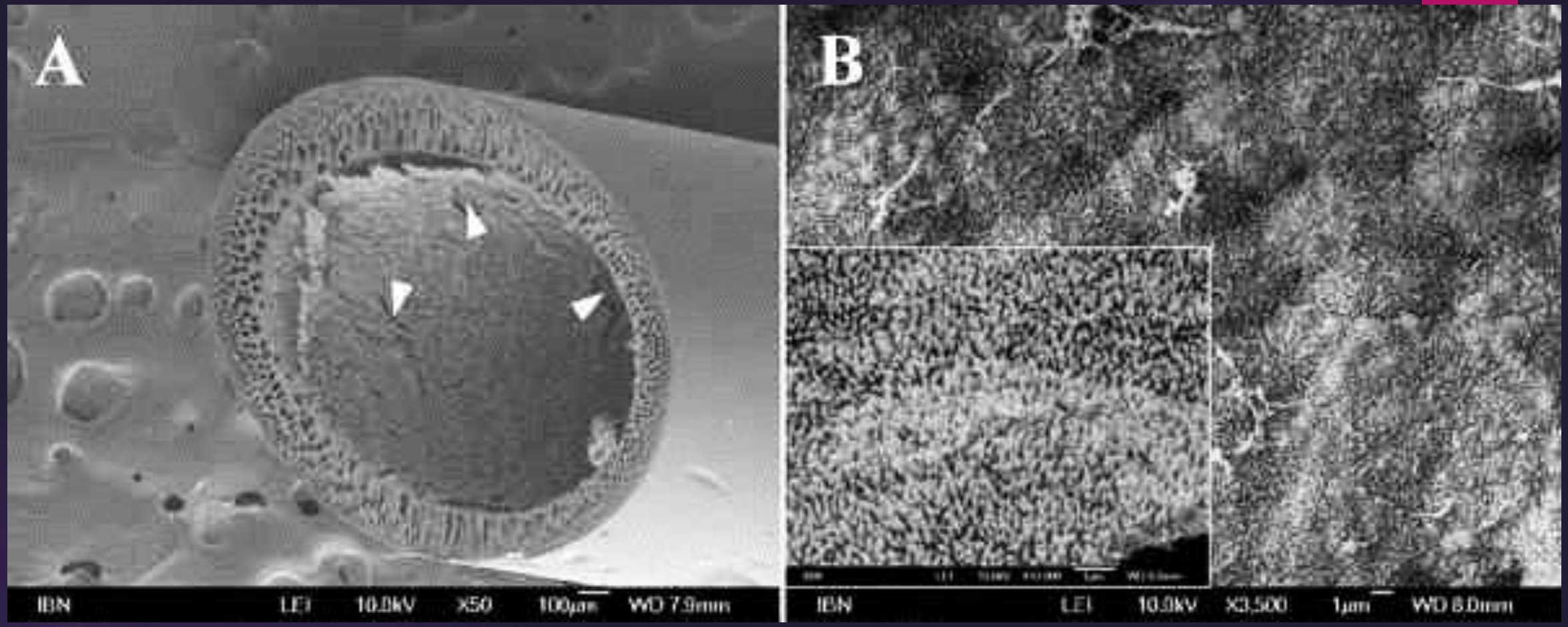
J. Cell. Mol. Med. Vol 17, No 4, 2013



HPTC grow in the bioreactor on the outer surfaces of the HFM, where they are exposed to the haemofiltrate. The blood flows in the lumina of the HFM

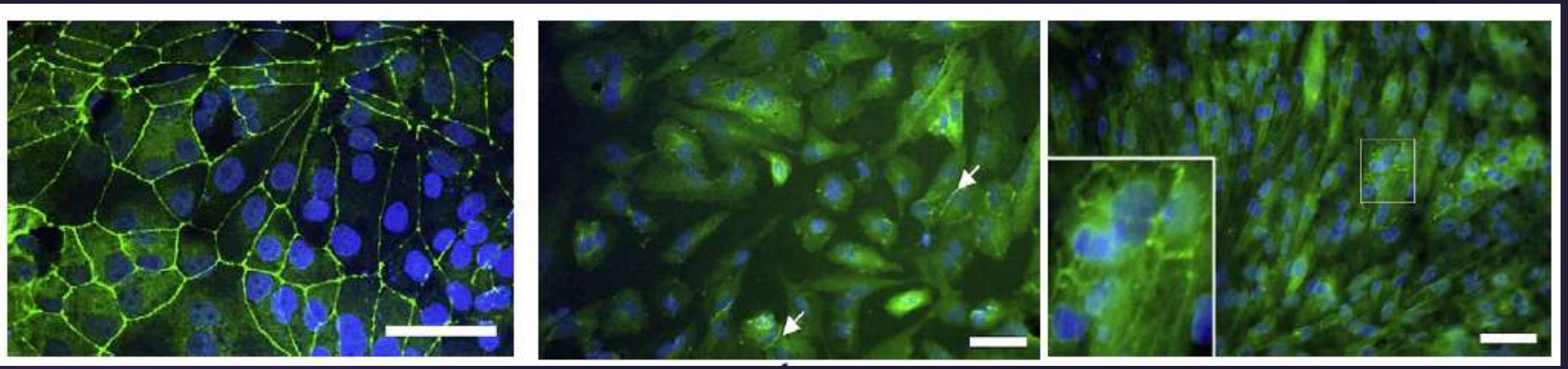
A novel design of bioartificial kidneys with improved cell performance and haemocompatibility





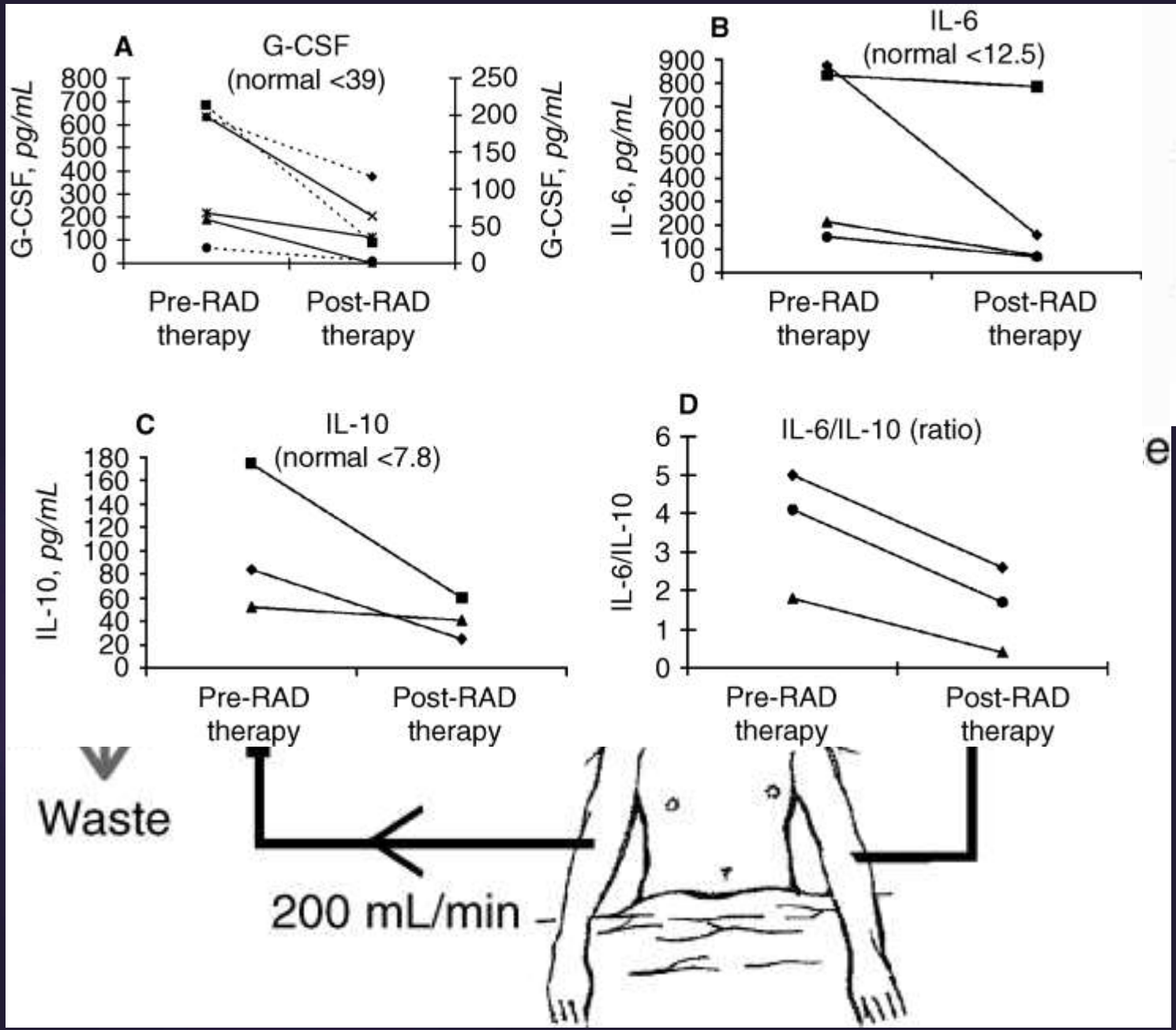
Madin-Darby canine kidney (MDCK) cells form a polarized epithelium on the inner surface of polyethersulfone/polyvinylpyrrolidone (PES/PVP) hollow fiber membranes. (a) Confluent monolayer of MDCK cells on the inner surface of a PES/PVP hollow fiber membrane

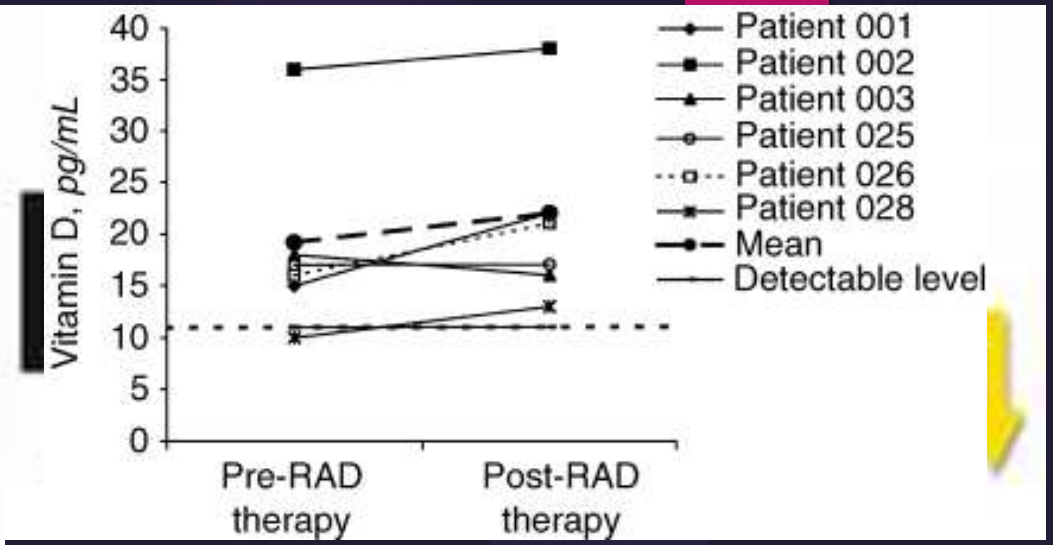
Fibrogenesis Tissue Repair v.3; 2010



Immunostaining for tight junction ZO-1 protein (green) of renal cells after one week incubation on coated flat synthetic membranes

HPTC on coated PES/PVP membrane





Initial clinical results of the bioartificial kidney containing human cells in ICU patients with acute renal failure

Humes etal Kidney International (2004) 66

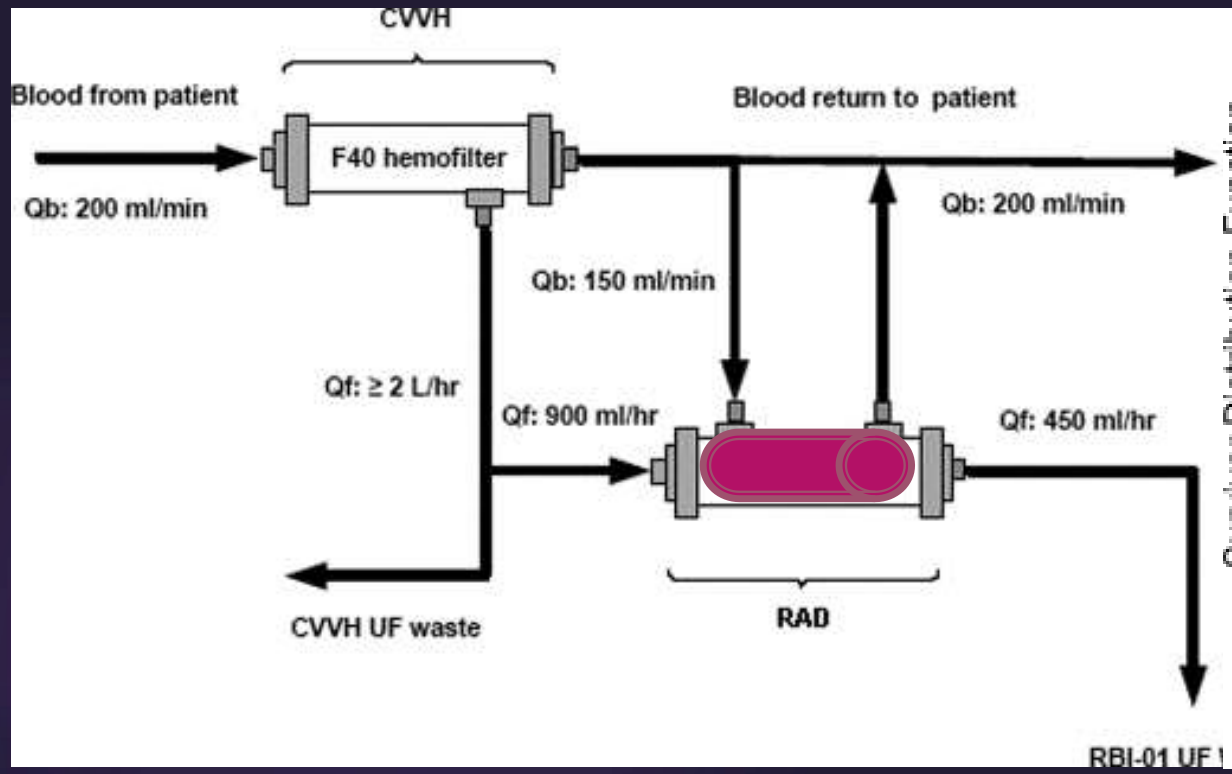
RAD : Clinical Trial All patients were critically ill with AKI and MOF

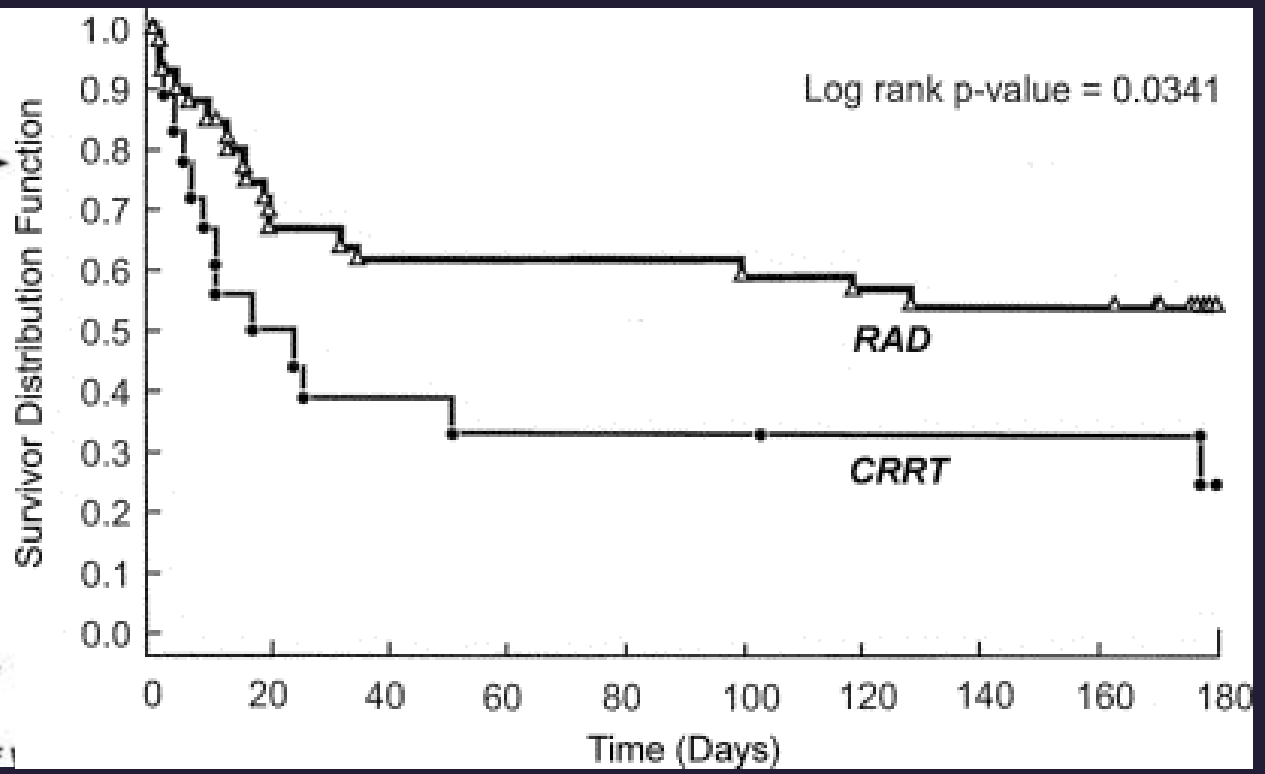
- initial study, consisting of 10 patients, demonstrated safe use of RAD therapy for up to 24 hours.
- Under RAD therapy, an actual mortality rate of 40% was demonstrated.
- RAD treatment resulted in significant declines in granulocyte-colony stimulating factor, interleukin (IL)-6, IL-10, and especially the IL-6/IL-10 ratio.
- RAD therapy improved the 28-day mortality rate from 61% in the conventional hemofiltration-treated control group to 34% in the RAD-treated group.
- the clinical trial was halted during the phase IIb interval analysis because of suboptimal clinical protocol design and several fabrication and manufacturing hurdles.

Humes,etal Blood Purif. 2004; 22: 60

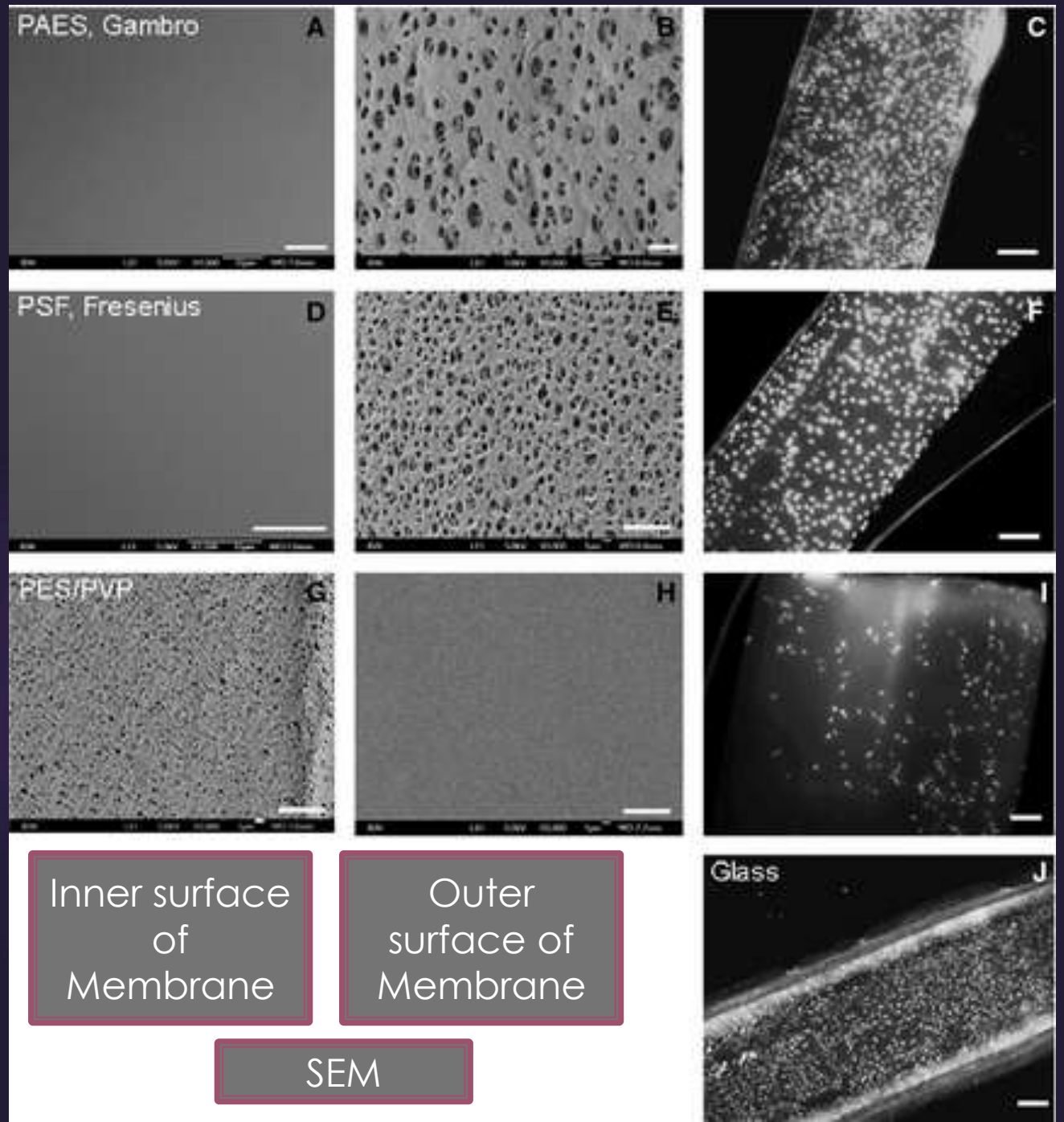
Tumlin etal J Am Soc Nephrol. 2008; 19

Efficacy and Safety of Renal Tubule Cell Therapy for Acute Renal Failure J Am Soc Nephrol. 2008 May; 19





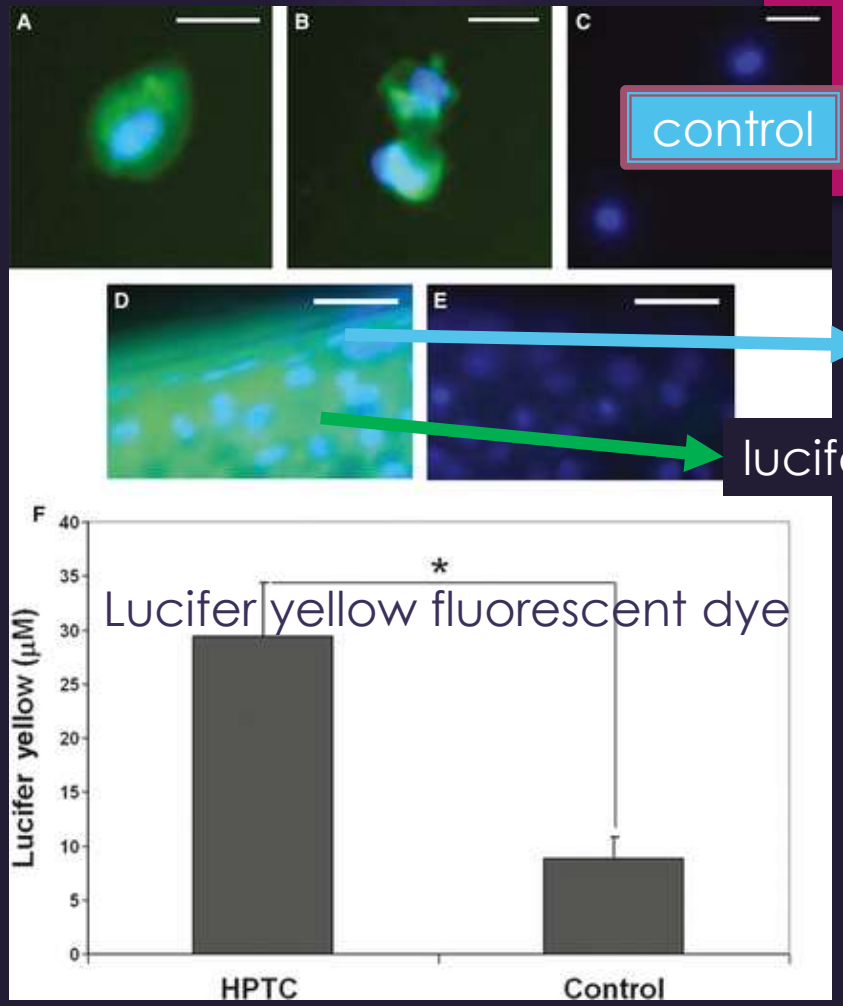
After the interim analysis, a corporate decision was made to discontinue enrollment in this study and proceed to the design of a confirmatory Phase II study



Inner surface of Membrane

Outer surface of Membrane

SEM



Baso-lateral cellular uptake and transport of lucifer yellow

J. Cell. Mol. Med. Vol 17, No 4, 2013

Cell Medicine, Vol. 4, pp. 33–43, 2012

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DOI: http://dx.doi.org/10.3727/215517912X653328
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Bioartificial Renal Epithelial Cell System (BRECS): A Compact, Cryopreservable Extracorporeal Renal Replacement Device

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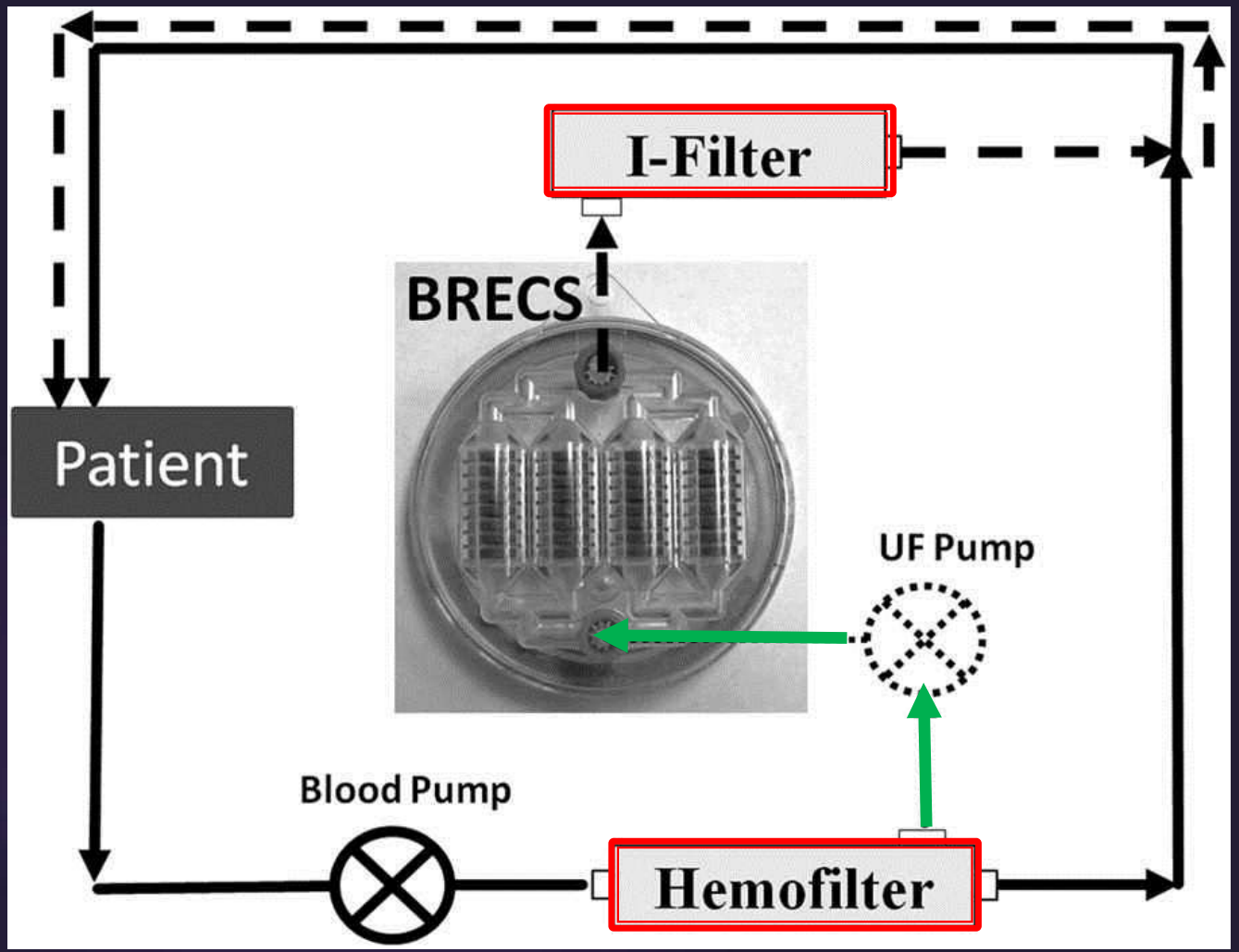
*Innovative BioTherapies, Inc., Ann Arbor, MI, USA
†Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, MI, USA
‡CytoPherx, Inc., Ann Arbor, MI, USA

Bioartificial Renal Epithelial Cell System (BRECS), ⇒ The BRECS was loaded with up to 20 cell-seeded porous disks

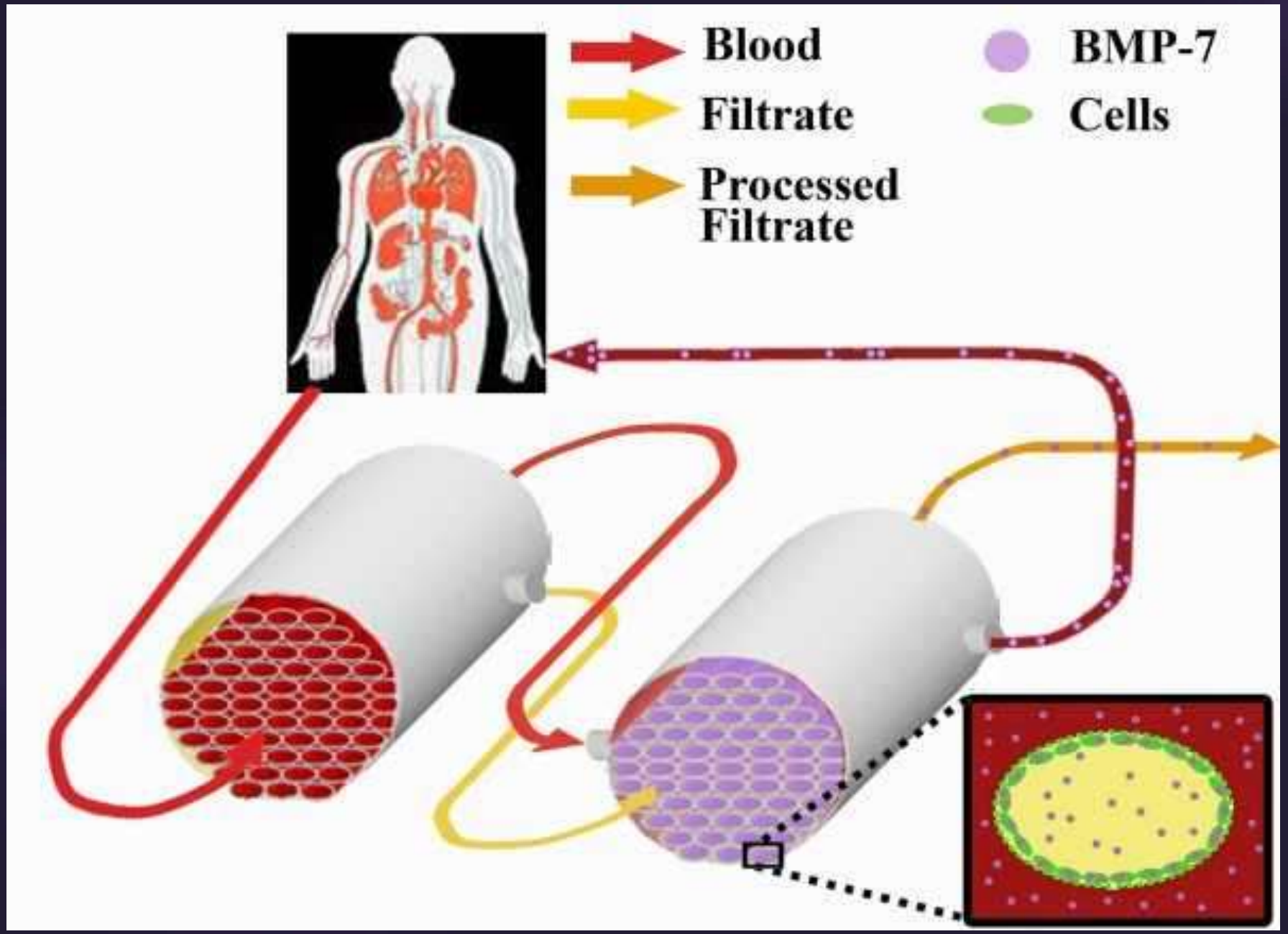
Once cells reached over 5×106 cells/disk for a total therapeutic dose of approximately 108 cells ⇒ BRECS was cryopreserved for storage at −80°C or −140°C.



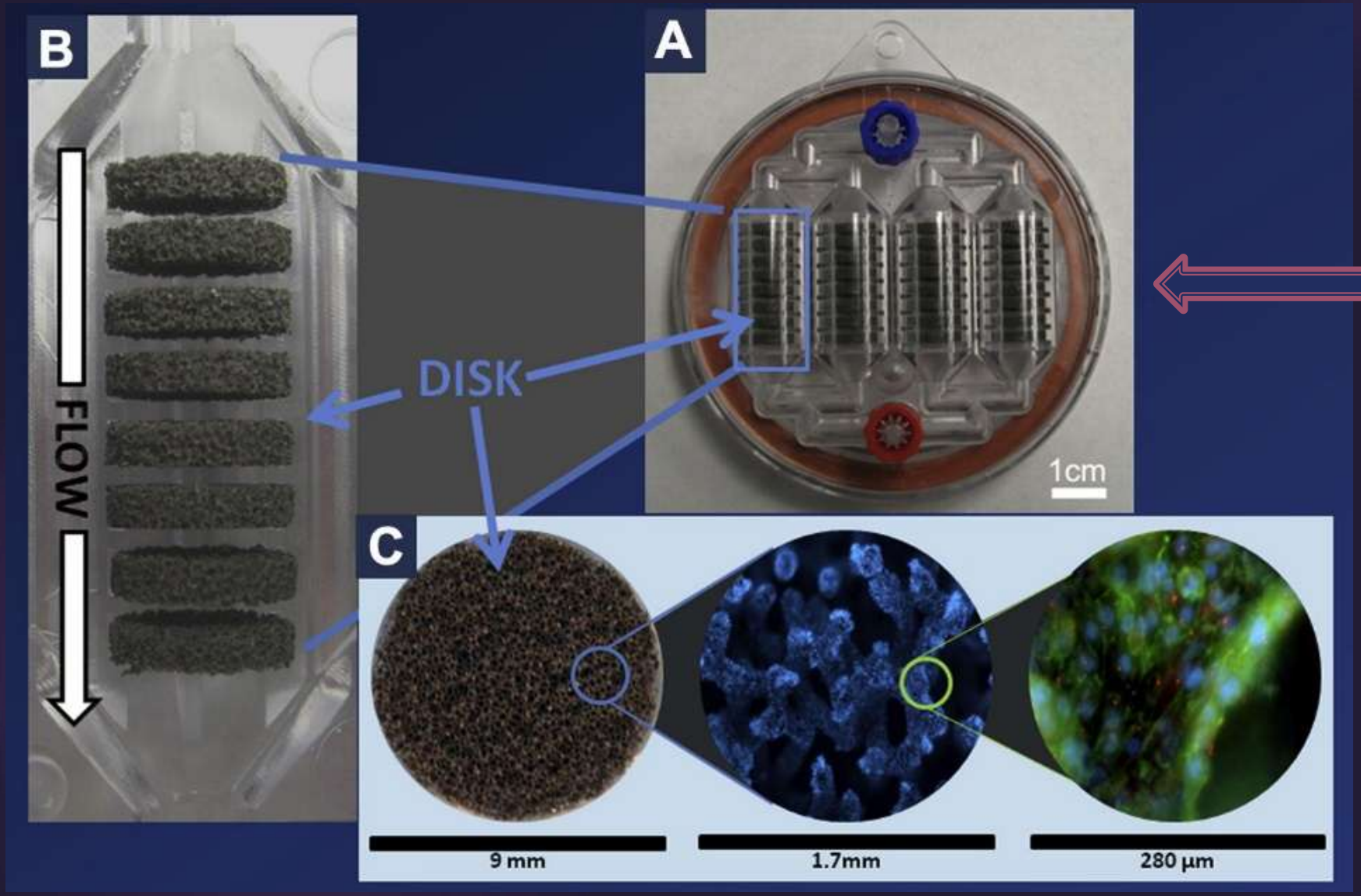
an immunoisolation filter (I-Filter), which protects the patient from any large molecular by-products and/or cells released by the BRECS that could cause an adverse reaction

65-kDa molecular weight cutoff

Translational Research 2014 163


Blood
Filtrate
Processed Filtrate
BMP-7
Cells

Translational Research 2014 163



The compact, freezable bioartificial renal epithelial cell system (BRECS) design

carbon disk stack channel

human renal epithelial cells derived from the enhanced propagation (EP) method of cell expansion

more than 5000 BRECS to be fabricated

Bioartificial kidney: steps toward clinical application

Hurdle	Solution status
FDA approval for first in human clinical trial testing.	Renal cell therapy has demonstrated safety and efficacy in preclinical (RAD and BRECS) and clinical studies (RAD).
Bioengineered cell system for mass production.	A compact BRECS has been developed for use in renal and inflammatory chronic and acute indications.
Bioengineered cell system capable for onsite, on-demand clinical use.	The BRECS can be cryopreserved for transport, storage, and reconstitution at clinical sites.

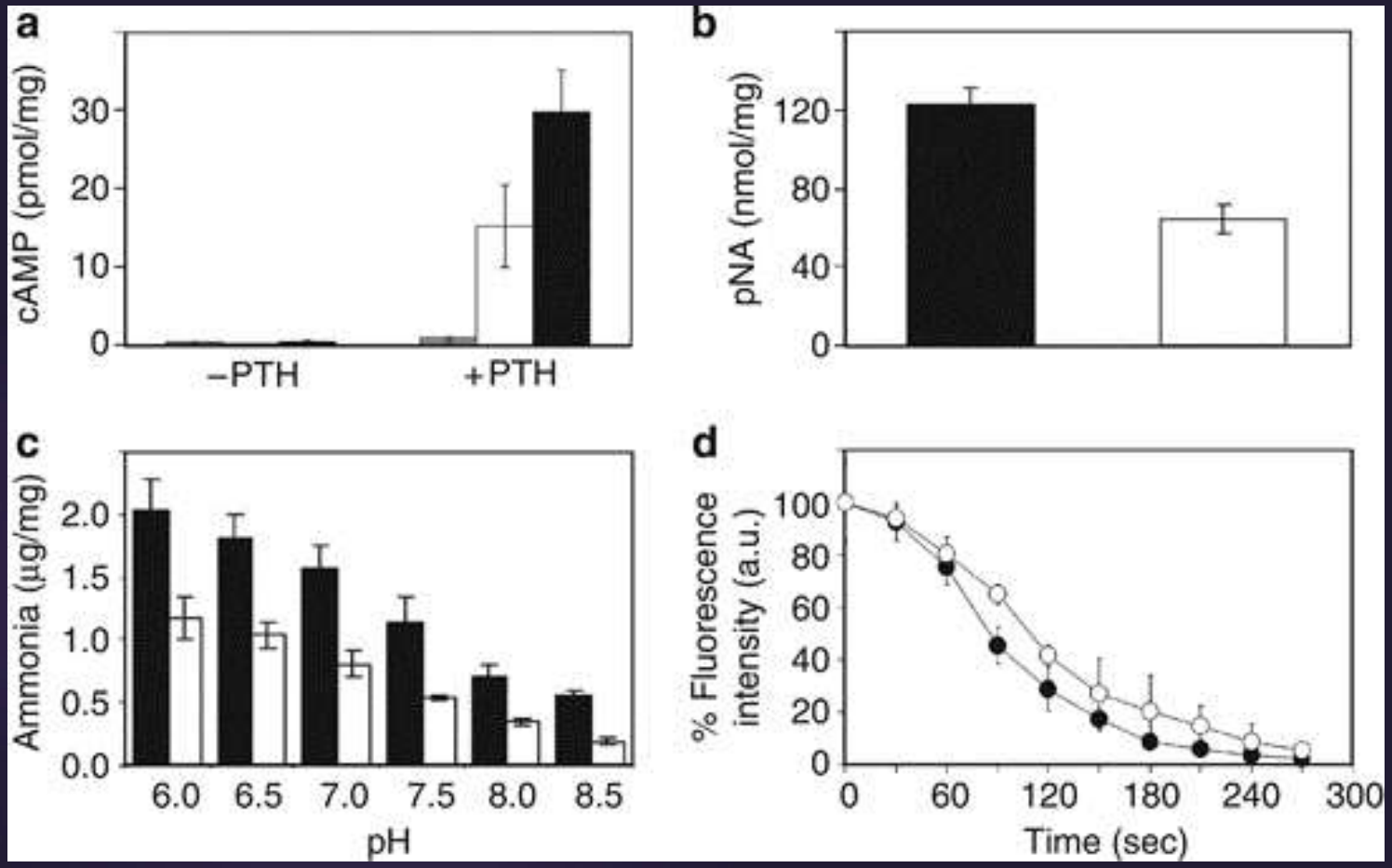
BRECS, bioartificial renal epithelial cell system

Translational Research ,April 2014Volume 163,

Toward a bioartificial kidney: will embryonic stem cells be the answer?

Kidney International (2013) 83, 543

Human embryonic stem cells differentiate into functional renal proximal tubular–like cells Narayanan etal Kidney International (2013) 83



(a) Response to parathyroid hormone (PTH)

(b) γ-glutamyl transferase (GGT) activity Measured by The concentration of p-nitroaniline (pNA) produced by the cells

(c) Ammonia production

(d) Water transport assay

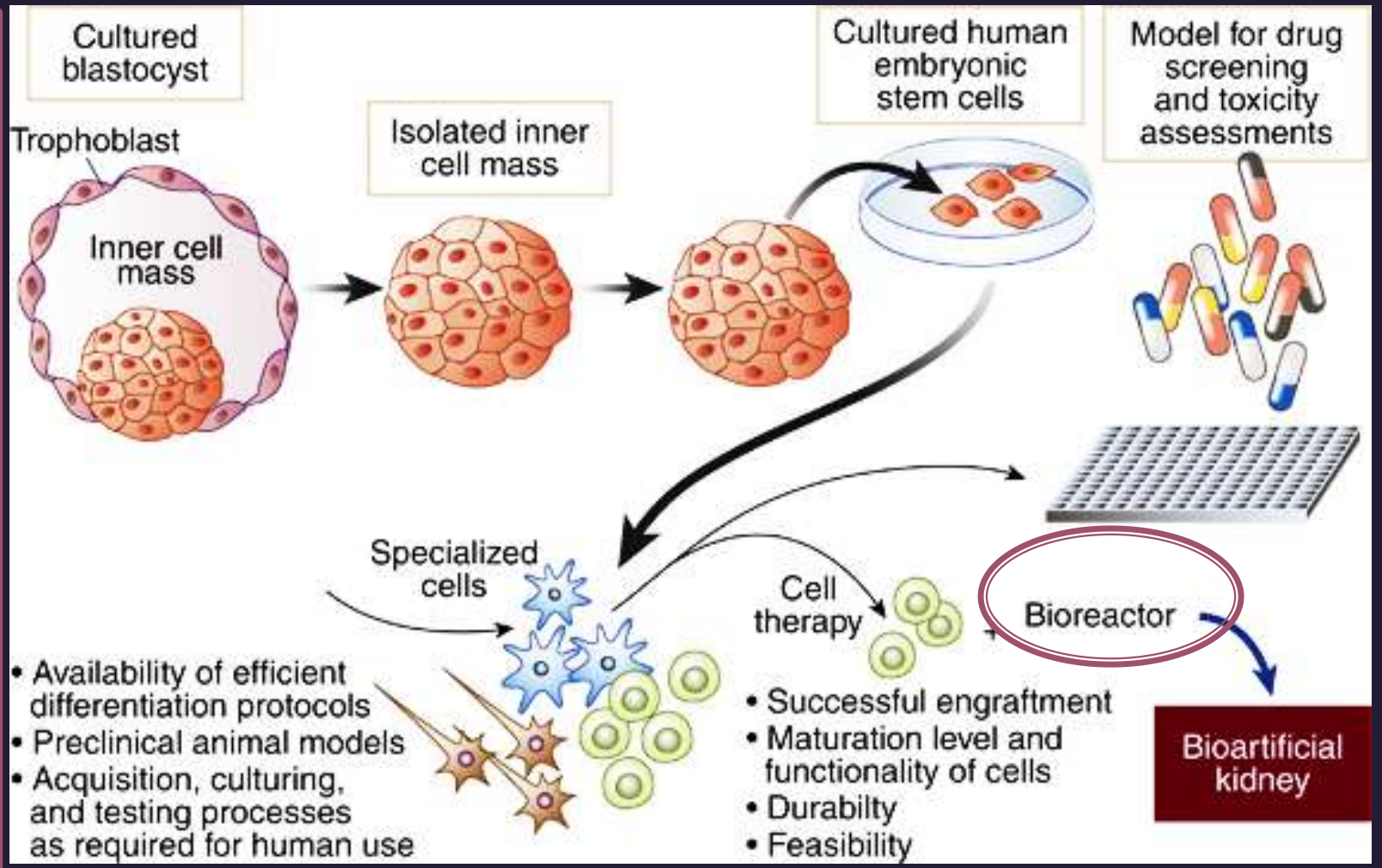
(HPTCs) (positive control, black bars)

Functional assays with differentiated human embryonic stem cells (hESCs) in static cultures

Toward a bioartificial kidney: will embryonic stem cells be the answer? Kidney International (2013) 83, 543

Problems
downregulation of several transporters, including apical and basolateral glucose transporters and organic acid, cationic, and peptide transporters.

Additionally, a proportion of these cells dedifferentiated in *in vivo* experiments, and hence future tumorigenesis studies and epigenetic characterization are important if clinical use is contemplated



CellR4 2015; 3 (1): e1401

Understanding the bioactivity of stem cells seeded on extracellular matrix scaffolds produced from discarded human kidneys: a critical step towards a new generation bio-artificial kidney

A. Petrosyan[1], G. Orlando[2,3], A. Peloso[2,4], Z. Wang[2], A.C. Farney[2], J. Rogers[2], R. Katari[2,3], S. Da Sacco[1], S. Sedrakyan[1], R.E. De Filippo[1], R.J. Stratta[2], S. Soker[3], L. Perin[1]

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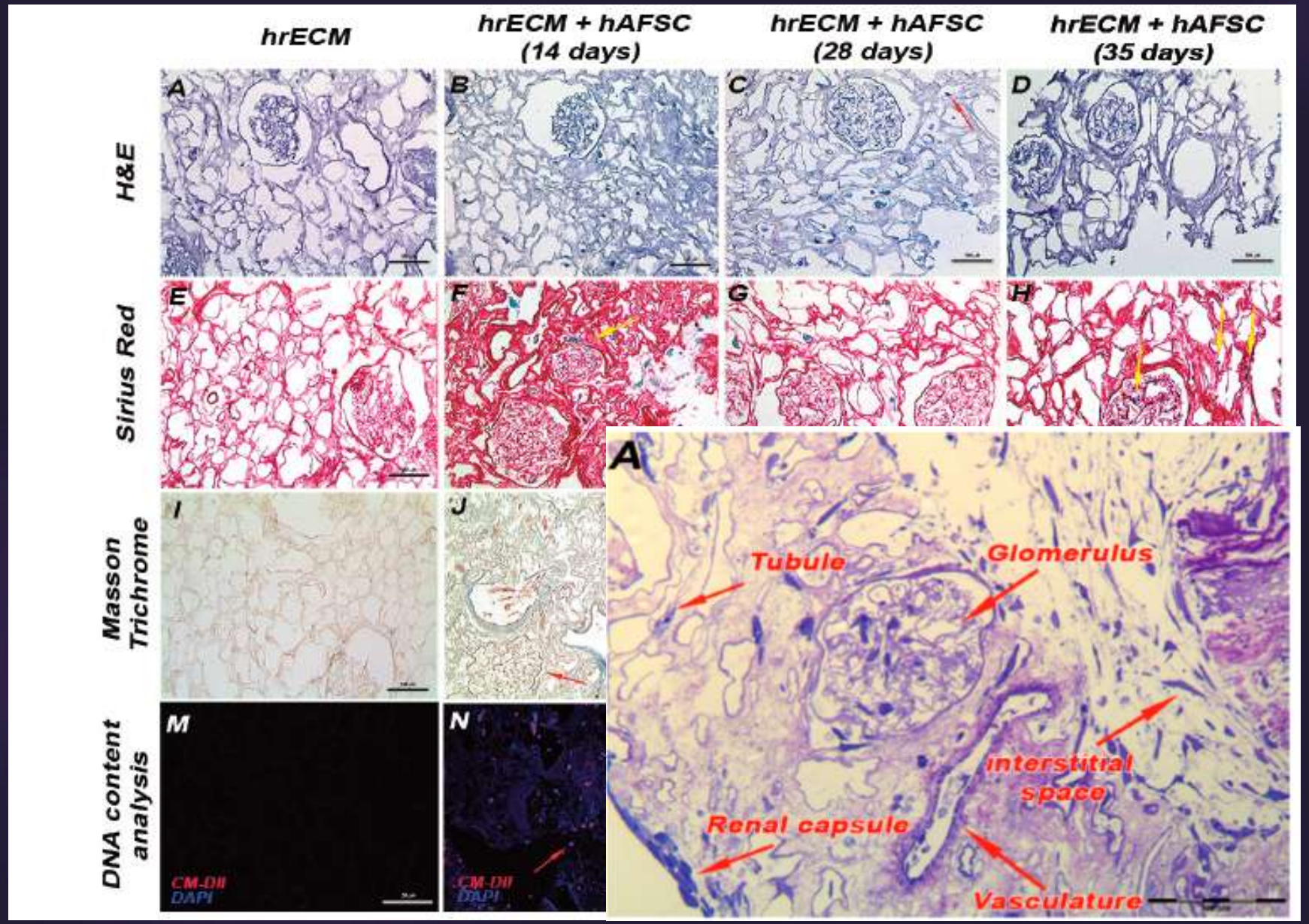
[4]General Surgery, Fondazione IRCCS Policlinico San Matteo Pavia and University of Pavia, Pavia, Italy

[5]Carolina Donor Services, Winston Salem, USA

Giuseppe Orlando and Astgik Petrosyan share the first Authorship



Histological analysis of Human Amniotic Stem cells (hAFSC) distribution over 14, 28, and 35 days after seeding on human renal extracellular matrix (hrECM)

CELLR4

CellR4 2015; 3 (1): e1401

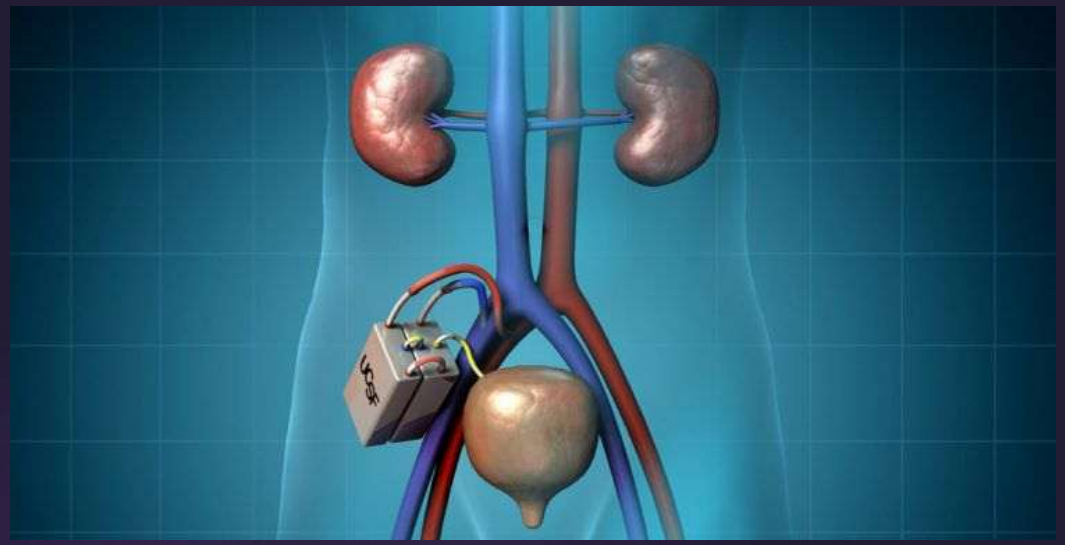
This model provides critical information which may be of importance in both kidney bioengineering and regeneration

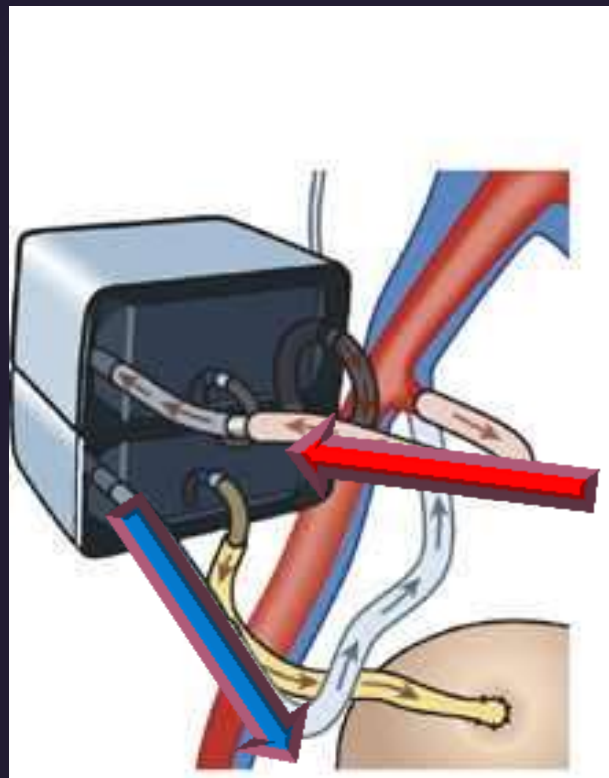
Implantable Device

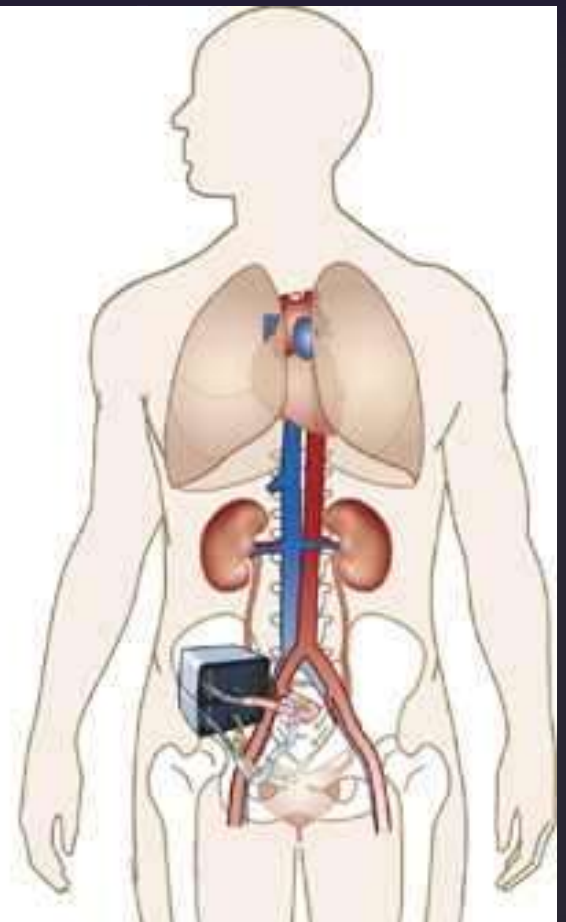
- University of California-San Francisco researchers unveiled a prototype model of the first implantable artificial kidney on September 2, 2010
- Received $3 million in funding as of October 1, 2012.
- Plan to go through human clinical trials by 2017.

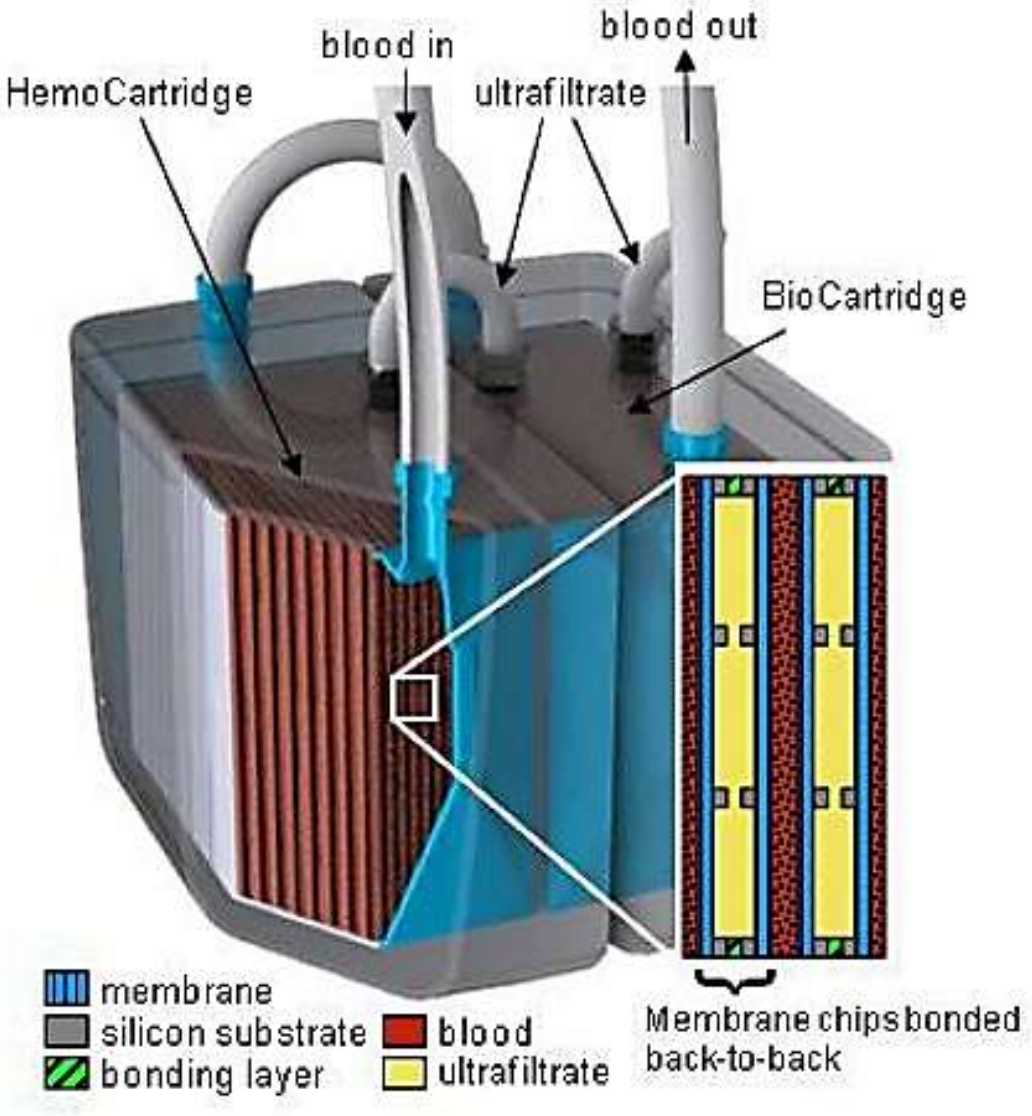
Implantable Device

University of California San Francisco









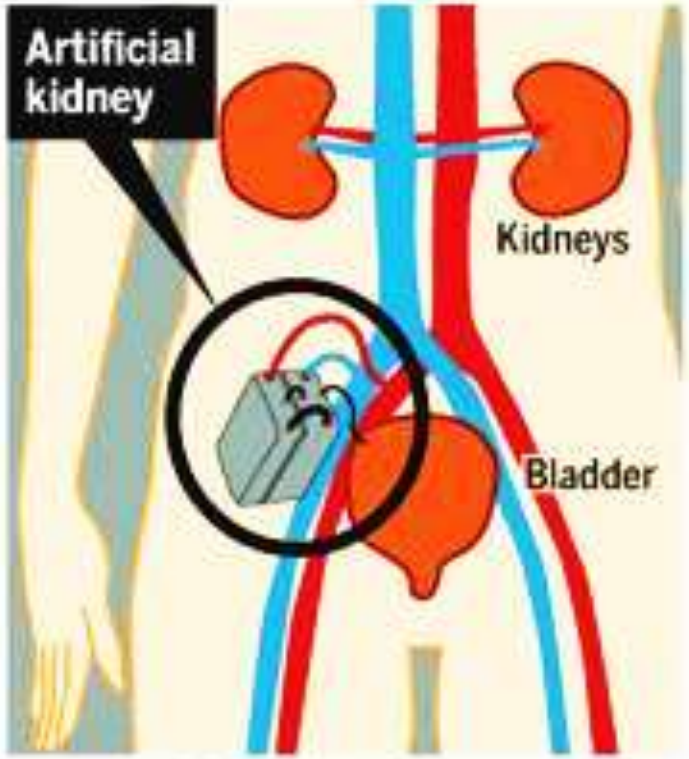
First compartment holds thousands of nano-scale filters remove toxins from the blood (Super efficient membranes)

A second compartment would hold live kidney cells that perform the other biological actions of a real kidney

Implantable Device

Implantable artificial kidney

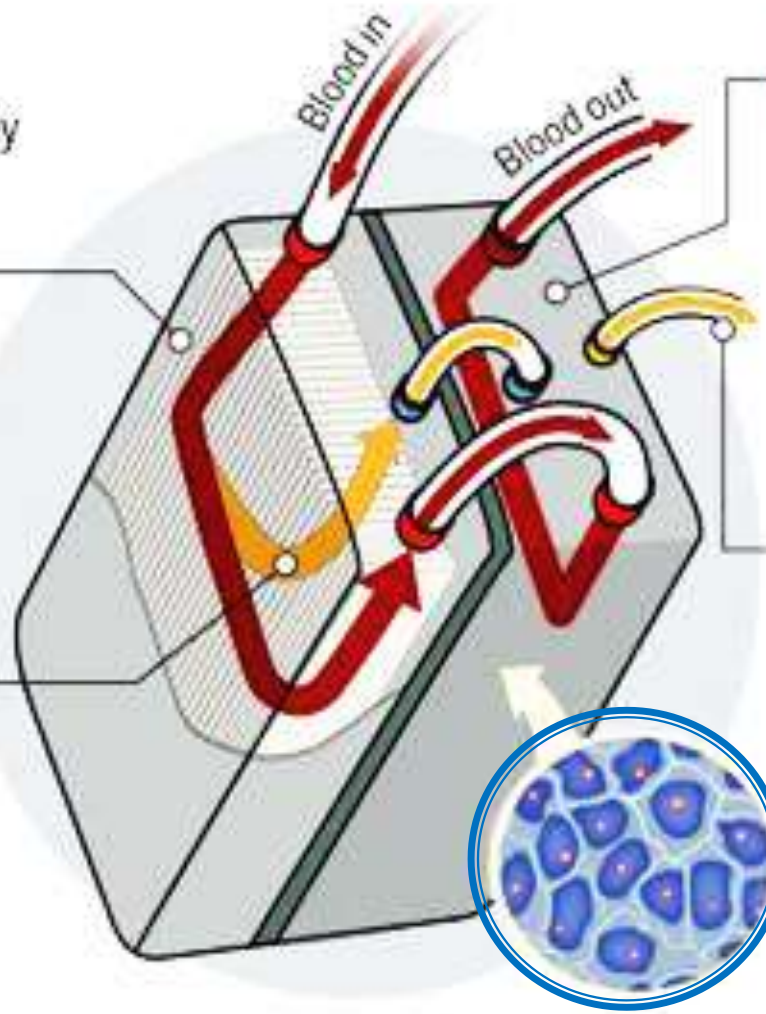
UC San Francisco is heading a team of researchers around the country who are working to create an implantable, artificial kidney the size of a coffee cup. The device consists of two chambers:



1 Hemofilter

The left chamber filters incoming blood with super-efficient membranes made with silicon nanotechnology.

Ultrafiltrate, separated from the blood, contains dissolved toxins, as well as water, sugars and salts.



2 Cell bioreactor

The right chamber contains live kidney cells that reabsorb much of the water, sugars and salts into the bloodstream.

The toxins and excess water are passed into the waste outlet connected to the bladder.

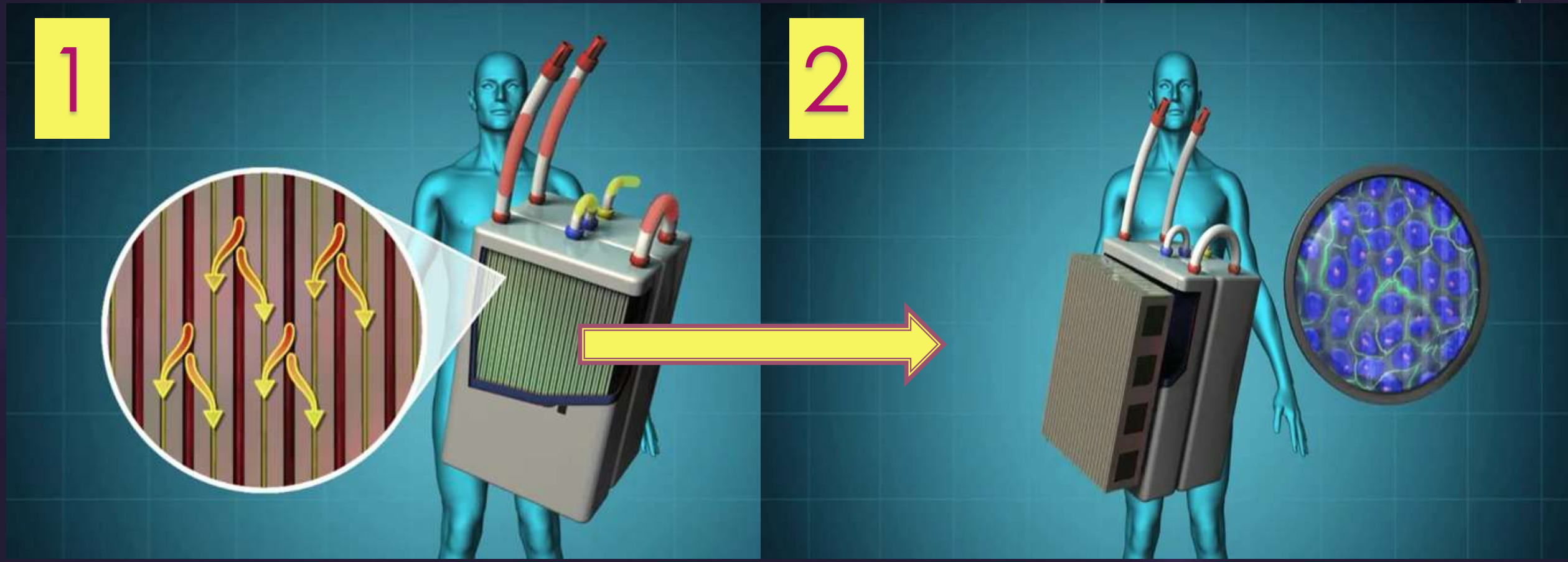
Kidney cells

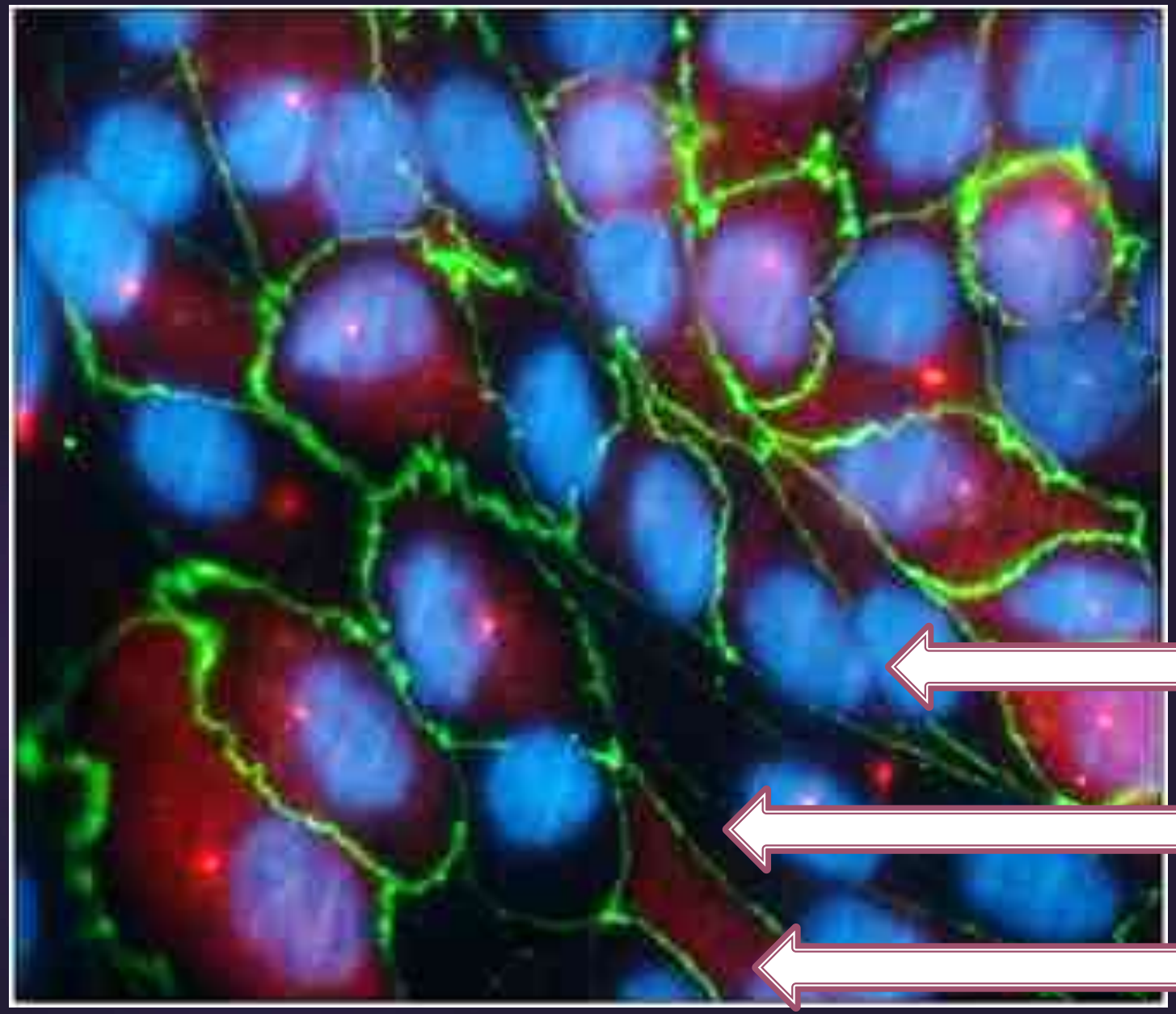
Source: UC San Francisco

BAY AREA NEWS GROUP

Implantable Device

Fast Tract FDA registration





Fluorescent microscopy image of human renal proximal tubule cells cultured from discarded renal transplant kidneys on silicon nanopore membranes.

Blue stain refers to cell nuclei,
green refers to tight junctions, and
red refers to cilia

Conclusion
Current status of bioartificial kidney

Journal of Biomedical Science and Engineering Vol.7 No.3(2014),

Although studies about cell therapy and wearable devices have not been conducted for quite a long-term period, it is promising that these new therapies will be available as options in the near future.

it is not a daydream that new therapeutic options such as Bioartificial kidney and wearable dialysis devices for ESRD patients will be applied in the routine clinical practice in nephrology area.

Thank you